DB Name	Query	Hit Count	Set Name
USPT,JPAB,EPAB,DWPI,TDBD	l10 and l11 and l12 and l14 and l17	11	L18
USPT,JPAB,EPAB,DWPI,TDBD	ampicillin	11779	L17
USPT,JPAB,EPAB,DWPI,TDBD	l10 and l11 and l12 and l14	16	L16
USPT,JPAB,EPAB,DWPI,TDBD	(phenylglycine amide) or (d-phenylglycine amide)	58	L15
USPT,JPAB,EPAB,DWPI,TDBD	(sulfuric acid) or h2so4	186645	L14
USPT,JPAB,EPAB,DWPI,TDBD	(sufuric acid) or h2so4	30723	L13
USPT,JPAB,EPAB,DWPI,TDBD	(d-phenylglycine) or phenylglycine	3390	L12
USPT,JPAB,EPAB,DWPI,TDBD	enzym$5	176652	L11
USPT,JPAB,EPAB,DWPI,TDBD	(6-aminopenicillanic acid) or 6-APA	1962	L10
JPAB,EPAB,DWPI	CN-1165032-$.did.	0	L9
JPAB,EPAB,DWPI	jp-05204120-$.did.	2	L8
JPAB,EPAB,DWPI	jp-02240026-$.did.	2	L7
JPAB,EPAB,DWPI	jp-05204120-$.did.	2	L6
JPAB,EPAB,DWPI	jp-5204120-$.did.	0	L5
JPAB,EPAB,DWPI	jp-2240026-$.did.	0	L4
JPAB,EPAB,DWPI	cn-1165032-$.did.	0	L3
JPAB,EPAB,DWPI	cn-1165032-$.did.	0	L2
JPAB,EPAB,DWPI	cn-1134306-$.did.	1	L1

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[illegible]

[illegible]

ABPL:
A novel Ampicillin ester of the general formula ##STR1## wherein R.sub.1 represents a hydrogen atom, a methyl group or an aryl group, and R.sub.2 represents a hydrogen atom or may be taken together with R.sub.1 to form a divalent carbon chain residue, or its acid addition salt.

ABPL:
The novel Ampicillin ester or its acid addition salt is prepared by (1) reacting a corresponding 6-N-acylamino penicillanic acid (II) or its salt with a compound of the formula ##STR2## wherein R.sub.1 and R.sub.2 are as defined above, and X is a halogen atom, or reacting a compound of the formula ##STR3## wherein R.sub.1 and R.sub.2 are as defined above, or its acid addition salt with a corresponding carboxylic acid (VI) or its reactive derivative, (2) thereafter, if required, when the resulting compound has the protected amino group or the group convertible to an amino group, deprotecting the protected amino group or converting said convertible group to an amino group, and (3) if further required, converting the product to an acid addition salt.

ABPL:
The present invention provides also an antibacterial agent comprising the novel Ampicillin ester and a method for the treatment of infectious disease.

BSPR:
This invention relates to novel Ampicillin esters, processes for their production, and to an antibacterial agent comprising such an Ampicillin ester.

BSPR:
Ampicillin [illegible] obtained by acylating the amino group of 6-aminopenicillanic acid (6-APA) with .alpha.-aminophenylacetic acid is a synthetic penicillin which is effective in oral administration. Absorption of Ampicillin from the digestive tract, however, is not sufficient, and this necessarily leads to administration of large dosages for obtaining the required concentration in blood, which in turn causes increased side-effects.

BSPR:
[illegible] Ampicillin, an attempt was made to convert Ampicillin to an ester-type derivative thereby improving its absorption from the intestinal tract. For example, Ampicillin [illegible] ester ([illegible]; see British Pat. No. [illegible]), and Ampicillin phthalidyl ester (Talampicillin; see British Pat. No. [illegible]) gives comparable blood Ampicillin concentrations in oral administration to those obtained by intramuscular administration.

BSPR:
It is an object of this invention to provide novel Ampicillin esters or their acid addition salts.

BSPR:
[illegible] novel and more beneficial

[illegible] orally administered [illegible] Ampicillin [illegible].

BSPR:
[illegible] Ampicillin esters which are [illegible] in gastric and intestinal juices, have better absorption from the intestinal tract, maintain a high concentration in blood over longer periods of time and are less toxic than known Ampicillin esters such as Talampicillin.

BSPR:
[illegible]

BSPR:
A further object of this invention is to provide a novel process for production of the novel Ampicillin esters.

BSPR:
According to this aspect, these objects and advantages are achieved by Ampicillin esters of the general formula ##STR4## wherein R.sub.1 represents a hydrogen atom, a methyl group or an aryl group, and R.sub.2 represents a hydrogen atom, or may be taken together with R.sub.1 to form a divalent chain [illegible], or their acid addition salts.

BSPR:
Specific examples of preferred Ampicillin esters of general formula (I) are:

BSPR:
The acid addition salts of these Ampicillin esters are, for example, salts of these esters with inorganic acids such as hydrochloric acid, hydrobromic acid, hydriodic acid and sulfuric acid, or salts of these with organic acids such as citric acid and tartaric acid.

BSPR:
Investigations of the present inventors have shown that the Ampicillin esters or the acid addition salts thereof have very desirable properties as pharmaceuticals.

BSPR:
Specifically, in oral administration, the Ampicillin esters of the invention are readily absorbed from the digestive tract, liberate Ampicillin in vivo, and maintain a high Ampicillin concentration in blood over long periods of time.

BSPR:
For example, thirty minutes after oral administration in mice, Ampicillin [illegible]-methyl-2-oxo-1,3-dioxolen-4-yl methyl ester hydrochloride and Ampicillin [illegible]-oxo-5-phenyl-1,3-dioxolen-4-yl methyl ester hydrochloride showed an Ampicillin concentration in blood about [illegible] times as high as that attained by the administration of Ampicillin and about 1.7 times as high as that attained by the administration of Ampicillin phthalidyl ester, and the high Ampicillin concentrations in blood are maintained over a long period of time. See Experiment 1 given hereinbelow.

BSPR:
[illegible] Ampicillin esters of this invention is [illegible] that while these Ampicillin esters readily undergo enzymatic hydrolysis in vivo, they have resistance to hydrolysis in gastric and intestinal juices.

BSPR:
[illegible] of Ampicillin [illegible]-methyl-2-oxo-1,3-dioxolen-4-yl methyl ester hydrochloride and Ampicillin [illegible]-oxo-5-phenyl-1,3-dioxolen-4-yl methyl ester hydrochloride in [illegible] and intestinal juices [illegible] phthalidyl ester. See Experiments [illegible] and [illegible]

[illegible] w.

BSPR:
[illegible] Ampicillin [illegible]

BSPR:
Experiments 1 to [illegible] are described below for demonstrating these advantages of the Ampicillin esters of the invention.

BSPR:
[illegible] equivalent to [illegible] ml of a [illegible] mg/ml aqueous solution of Ampicillin . The blood was taken from the experimental animals periodically, and the concentration of Ampicillin in the serum was measured by a bioassay method. The blood Ampicillin level ratio was calculated from the following equation. ##EQU1##

BSPR:
The results given in Table 1 clearly show that the compounds of the invention show a high blood Ampicillin level over a longer period of time than the known [illegible].

BSPR:
Prodrugs such as Ampicillin pivaloyloxymethyl ester or Ampicillin phthalidyl ester have been known as orally administrable Ampicillin. The ester group of the Ampicillin ester of the invention (i.e., (2-oxo-1,3-dioxolen-4-yl)methyl group) is shown by a formula below in comparison with those of the known prodrugs.

BSPR:
It is clear therefore that the ester group of the Ampicillin ester of the invention quite differs from those of the known Ampicillin esters. It is surprising that the Ampicillin esters of the present invention have the aforesaid excellent properties as pharmaceuticals over these known Ampicillin esters.

BSPR:
According to one process of the invention, the Ampicillin ester or its acid addition salt of the invention can be produced by reacting a compound of the general formula ##STR4## wherein A represents a protected amino group or a group convertible to an amino group, or its salt at the carboxyl group with a compound of the general formula ##STR5## wherein R.sub.1 and R.sub.2 are as defined above, and X represents a halogen atom, and if required, when the resulting compound has the protected amino group or the group convertible to an amino group, eliminating the protecting group from the protected amino [illegible], and if further required, converting the product to its acid addition salt.

BSPR:
A compound corresponding to general formula (II) in which A is a free amino group is a compound well known as Ampicillin and readily available commercially.

BSPR:
Accordingly, the compound of general formula (II) can be produced by converting the free amino group of Ampicillin to the group A (in this case, the group A is desirably a protected amino group).

BSPR:
The compound of general formula (II) can also be produced by reacting [illegible] or its salt at the carboxyl group with a carboxylic [illegible] ##STR6## wherein A is as defined hereinabove, or its [illegible]

[illegible] of formula (II) are described, for example, in U.S. Pat. No. [illegible].

BSPR:
Thus, the Ampicillin ester of general formula (I) or its acid addition salt is formed. The acid addition salt is prepared by reacting the Ampicillin ester having a free amino group of general formula (I) with an acid, for example, an inorganic acid such as hydrochloric acid, hydrobromic acid, hydriodic acid and [illegible] or an organic acid such as citric acid or tartaric acid.

BSPR:
According to preferred embodiments of the process of the invention, there are provided a process for producing the Ampicillin ester of general formula (I) or its acid addition salt, which comprises reacting a compound of general formula (II) in which A is a Schiff base group or an enamine group with the compound of general formula (III), and converting the Schiff base group or the enamine group of the product to an amino group, and thereafter if required, converting the product to its acid addition salt; and a process for producing a mineral acid salt (e.g., hydrochloride) of the Ampicillin ester of general formula (I) which comprises reacting a compound of general formula (II) in which A is an amino group in the form of a mineral acid such as hydrochloride, with the compound of general formula (III).

BSPR:
According to another process provided by the invention, the Ampicillin ester of general formula (I) or its acid addition salt can be produced by reacting a compound of general formula ##STR13## wherein R.sub.1 and R.sub.2 are as defined above, or its acid addition salt, with a carboxylic acid of the general formula ##STR14## wherein A is as defined above, or its reactive derivative at the carboxyl group; thereafter, if required, when the resulting compound has the protected amino group or the group convertible to an amino group, eliminating the protective group from the protected amino group, or converting said convertible group to an amino group, and if further required, converting the product to an acid addition salt thereof.

BSPR:
The compound of general formula (V) can be produced by reacting 6-aminopenicillanic acid or its salt at the carboxyl group with the compound of general formula (III); or by reacting 6-protected aminopenicillanic acid or its salt at the carboxyl group with the compound of general formula (III) and then converting the protected amino group of the reaction product to an amino group.

BSPR:
The former can be performed preferably by reacting 6-aminopenicillanic acid or its salt at the carboxyl group with an equimolar amount, or a molar excess, of the compound of general formula (III) in an inert organic solvent such as tetrahydrofuran, dioxane or acetone in the optional presence of a base (when 6-aminopenicillanic acid is used, the presence of a base is preferred) at a temperature of from about 0.degree. C. to room temperature.

BSPR:
The latter can be performed preferably by reacting 6-protected aminopenicillanic acid such as 6-aminopenicillanic acid having the amino group at the 6-position protected with an acyl group or trityl group, or 6-aminopenicillanic acid having the amino group at the 6-position protected as a Schiff base, or its salt at the carboxyl group, for example 6-phenylacetylaminopenicillanic acid (benzylpenicillin), with the compound of general formula (III) under the same conditions as in the first-mentioned process, thereafter reacting the resulting 6-protected aminopenicillanic acid ester with phosphorus pentachloride and a lower alcohol such as methanol at [illegible] in the presence of a basic compound such as N-methylmorpholine, pyridine and triethylamine, and thereafter causing water [illegible] the resulting [illegible] to hydrolyze it.

BSPR:
[illegible] group, the protecting group is removed from the protected amino group, or the [illegible] group is converted to an amino group and if desired, the product is converted to its acid addition salt. Thus, the Ampicillin ester of general formula (I) or its acid addition salt is formed.

BSPR:
[illegible] which A is a Schiff base group or an enamine group with the compound of general formula (V), thereafter converting the Schiff base group or the enamine group "A" of the resulting compound to an amino group and if required, converting the product into its acid addition salt; and a process for producing an acid addition salt, such as a hydrochloride, of the Ampicillin ester of general formula (I) which comprises reacting a compound of general formula (II) in which A is in the form of an acid addition salt such as a hydrochloride with the compound of general formula (V).

BSPR:
After the reaction, the Ampicillin of general formula (I) or its acid addition salt can be isolated and purified in a customary manner.

BSPR:
The Ampicillin ester of general formula (I) or its pharmaceutically acceptable acid addition salt is converted back to Ampicillin in vivo when administered to an animal. Accordingly, this invention also provides an antibacterial agent comprising the Ampicillin ester of general formula (I) or its pharmaceutically acceptable acid addition salt as an active ingredient.

BSPR:
The antibacterial agent of this invention may consist only of the Ampicillin ester of general formula (I) or its pharmaceutically acceptable acid addition salt, or a mixture of it with a pharmaceutically acceptable carrier.

BSPR:
The pharmaceutically acceptable carrier may be those carriers which can be used in formulating Ampicillin. Examples are starch, lactose, hydroxypropyl cellulose, crystalline cellulose, magnesium stearate, and calcium stearate.

BSPR:
The antibacterial agent of this invention is administered to man and other animals in a dose of [illegible] mg/kg body weight/day calculated as the Ampicillin ester (I) or its pharmaceutically acceptable salt.

BSPT:
Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester (R.sub.1 =methyl, R.sub.2 =hydrogen),

BSPT:
Ampicillin (2-oxo-1,3-dioxolen-4-yl)methyl ester (R.sub.1 and R.sub.2 =hydrogen),

BSPT:
Ampicillin (2-oxo-5-phenyl-1,3-dioxolen-4-yl)methyl ester (R.sub.1 =phenyl, R.sub.2 =hydrogen),

BSPT:
Ampicillin [illegible] hexen-1-yl ester (R.sub.1 and R.sub.2 [illegible] --R.sub.1 --R.sub.2 --), and

BSUM:
[illegible]

BSUM:
A. Ampicillin 5-methyl-2-oxo-1,3-dioxolen-4-yl methyl ester hydrochloride (compounds of the invention)

BSUM:
B. Ampicillin 2-oxo-5-phenyl-1,3-dioxolen-4-yl methyl ester hydrochloride (compounds of the invention)

BSUM:
C. Ampicillin phthalidyl ester hydrochloride (a known compound used as a control; see British Pat. No. [illegible])

BSUM:
D. Ampicillin trihydrate (control)

BSUM:
TABLE 1

Item	Test time of blood taking (min.) Compound	15	30	60	90	120	180
Compounds of the invention	A	2.6	2.9	2.1	1.8	1.5	1.3
	B	[illegible]	[illegible]	2.4	1.7	1.2	[illegible]
Known compound	C	3.0	1.8	1.4	1.1	[illegible].9	1.5
Control compound	D	1.0	1.0	1.0	1.0	1.0	1.0

(Ampicillin level ratio)

BSTL:
Ester group
Ampicillin pivaloyloxy methyl ester ##STR5## Ampicillin phthalidyl ester ##STR6## Ampicillin ester of the invention ##STR7##

CHIR:
Ampicillin trihydrate ([illegible] mg) was dispersed in [illegible] ml of dimethyl formamide, and [illegible] mg of potassium hydrogen carbonate was added. The mixture was cooled to [illegible].degree. C. and stirred. Benzaldehyde ([illegible] ml) was added, and the mixture was stirred at [illegible].degree. C. for 1.5 hours. Then, 125 mg of potassium hydrogen carbonate and 32[illegible] mg of 4-bromomethyl-5-phenyl-1,3-dioxolen-2-one were added, and the mixture was further stirred at 0.degree. C. for 3 hours.

DEPR:
Water ([illegible] ml) was added, and the mixture was concentrated under reduced pressure to distill off [illegible]. The aqueous layer was repeatedly washed with ethyl acetate, and saturated with sodium chloride. The separated oily substance was extracted with [illegible] ml of methylene chloride, washed with a saturated aqueous solution of sodium chloride and dried over anhydrous sodium sulfate. The dried organic layer was concentrated until the amount of methylene chloride decreased to one half. Isopropyl alcohol (30 ml) was added, and the mixture was again concentrated under reduced pressure to give a colorless solid. The solid was collected by filtration, and washed successively with isopropyl alcohol and ether to give 320 mg (yield 46.4[illegible]) of Ampicillin 2-oxo-5-phenyl-1,3-dioxolen-4-yl methyl ester hydrochloride as a [illegible].

DEPR:
The resulting Ampicillin ester hydrochloride was incubated in 40[illegible] mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

DEPR:
Ampicillin trihydrate [illegible] was dispersed in [illegible] ml of dimethyl formamide, and [illegible] was added. The mixture was [illegible]. [illegible] was added. The mixture was stirred at [illegible]. Then, [illegible] potassium hydrogen carbonate and [illegible]

[illegible] 4-[illegible] methyl-[illegible]-1,3-[illegible] was added, and the mixture was [illegible]. After the reaction, the reaction mixture was poured into [illegible] water. The precipitated [illegible] was extracted with [illegible] ml of ethyl acetate. The [illegible] layer was washed with [illegible] ml of water three times, and dried over anhydrous magnesium sulfate. The ethyl acetate was distilled off under reduced pressure to give a yellow syrup. The resulting syrupy residue was dissolved in 4 ml of acetonitrile and the solution was adjusted to pH [illegible] with dilute hydrochloric acid. The solution was then stirred at [illegible] for [illegible] minutes. Water [illegible] ml was added, and the [illegible] was distilled off under reduced pressure. The aqueous layer was [illegible] with ethyl acetate, and then saturated with sodium chloride. [illegible] was extracted with [illegible] ml of methylene chloride, and washed with [illegible] aqueous [illegible] chloride. The methylene chloride layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to one half of its volume. To the solution isopropyl alcohol [illegible] ml was added, and the mixture was again concentrated under reduced pressure to give a colorless amorphous solid.

DEPR:
The solid was collected by filtration and washed with isopropyl alcohol and ether to give [illegible] mg (yield [illegible]) of Ampicillin [illegible]-methyl-[illegible]-1,3-[illegible]-4-yl)methyl ester hydrochloride as a colorless amorphous solid. The product had the following properties.

DEPR:
The resulting Ampicillin ester hydrochloride was incubated in 40% mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

DEPR:
By the same method as shown in Example 1, [illegible], 250 mg of Ampicillin [illegible],4-[illegible]-[illegible]hexenyl) ester hydrochloride was obtained as a colorless amorphous solid from [illegible] g of Ampicillin trihydrate and 1 g of [illegible]-1,2-[illegible]cyclohexene (yield [illegible]).

DEPR:
The resulting Ampicillin ester hydrochloride was incubated in 40% mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

DEPR:
Ampicillin trihydrate [illegible] was dispersed in [illegible] ml of dimethyl formamide, and [illegible] mg of potassium hydrogen carbonate was added. The mixture was cooled to [illegible].degree. C., and 1 ml of [illegible]aldehyde was added. The mixture was stirred at [illegible].degree. to [illegible].degree. C. for 3 hours. To the mixture were added 500 mg of potassium hydrogen carbonate and 1 g of 4-bromomethyl-1,3-dioxolen-2-one, and the mixture was stirred at [illegible].degree. to [illegible].degree. C. for [illegible] hours.

DEPR:
Water [illegible] ml was added, and the mixture was concentrated under reduced pressure to remove acetonitrile. The aqueous layer was repeatedly washed with ethyl acetate, and saturated with sodium chloride to precipitate an orange gum-like substance. The aqueous layer was removed by decantation. The gum-like substance was dissolved in methanol, decolorized with activated carbon, cooled [illegible].degree. C., and poured into vigorously stirred ether to precipitate a pale orange solid. The solid was collected by filtration, and washed with a mixture of ether and methanol to give [illegible] mg (yield [illegible]) of Ampicillin [illegible]-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride as a pale orange amorphous solid.

DEPR:
The resulting Ampicillin ester hydrochloride was incubated in 40% mouse blood in pH 7.4 phosphate buffer at 37.degree. C. for 10 minutes, and then subjected to bioautography. It was found to be completely converted to Ampicillin.

DEPR:
[illegible] the hydrochloride of the [illegible] was formed. Phosphorus pentachloride [illegible] was added, and the mixture was stirred at [illegible].degree. to [illegible].degree. C. for 4 hours. The solid precipitated was collected by filtration, and repeatedly washed with methylene chloride to give 13.1 g (yield [illegible]) of D-(-)-phenylglycyl chloride hydrochloride was a colorless amorphous solid.

DEPR:
[illegible]

DEPR:
After the reaction, the solid was separated by filtration, and the filtrate was concentrated under reduced pressure. The resulting syrup was dissolved in water, and washed with ethyl acetate. The aqueous layer was saturated with sodium chloride, and the separated oily substance was extracted with methylene chloride. The extract was washed with a saturated aqueous solution of sodium chloride and concentrated until the amount of methylene chloride decreased to half. Upon addition of isopropyl alcohol, a colorless solid was precipitated. The solid was collected by filtration and washed with isopropyl alcohol and ether to give 132 mg (yield 54%) of Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride as an amorphous solid.

DEPR:
From [illegible] of the resulting ester hydrochloride and 95 mg of D-(-)-phenylglycyl chloride hydrochloride, 14[illegible] mg (yield 56[illegible]) of Ampicillin [illegible]-phenyl-1,3-dioxolen-4-yl)methyl ester hydrochloride was obtained as a colorless amorphous solid.

DEPR:
Five grams of (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl 6-aminopenicillanate p-toluenesulfonate was suspended in 300 ml of ethyl acetate. To the suspension was added at [illegible].degree. C. [illegible] ml of a [illegible] aqueous solution of sodium hydrogen carbonate [illegible] with ice. The mixture was vigorously stirred. The ethyl acetate layer was separated, washed with ice water, dried at 0.degree. C. over anhydrous magnesium sulfate, and concentrated under reduced pressure to give a pale yellow syrup. The syrup was dissolved in 50 ml of methylene chloride. The solution was cooled to 0.degree. C., and 1 g of potassium hydrogen carbonate and 2.1 g of D-(-)-phenylglycyl chloride hydrochloride were added, and the mixture was stirred at 0.degree. C. for 4 hours. After the reaction, the insoluble material was separated by filtration, and the filtrate was concentrated under reduced pressure. The resulting syrup was dissolved in water and washed with ethyl acetate. The aqueous layer was saturated with sodium chloride. The separated oily substance was extracted with methylene chloride, washed with a saturated aqueous solution of sodium chloride and dried over anhydrous sodium sulfate. The dried solution was concentrated under reduced pressure until the amount of methylene chloride decreased to one half. [illegible] was added, and the mixture was again concentrated under reduced pressure to give a colorless solid. The solid was collected by filtration, and washed with ether [illegible] (yield [illegible]) of Ampicillin [illegible]-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride as a colorless amorphous solid.

[illegible]
[illegible]

DEPR:
The [illegible] hydrochloride, crystalline cellulose and lactose were mixed, and an ethanol solution of hydroxypropyl cellulose was added. They were kneaded and dried. To the dried mixture was added magnesium stearate. They were mixed and tabulated to form a tablet.

DEPC:
(1) Production of Ampicillin (2-oxo-5-phenyl-1,3-dioxolen-4-yl)methyl ester hydrochloride

DEPC:
(2) Production of Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride

DEPC:
(2) Production of Ampicillin (2,3-carbonyldioxy-2-[illegible])methyl ester hydrochloride

DEPC:
(2) Production of Ampicillin (2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride

DEPC:
(4) Production of Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride

DEPC:
Production of Ampicillin (2-oxo-5-phenyl-1,3-dioxolen-4-yl)methyl ester hydrochloride

DEPC:
(1) Production of Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride

DETL:
(1) Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride 356.7 mg Lactose 38.3 mg Magnesium stearate 5.0 mg 400 mg in total

DETL:
(2) Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride [illegible] mg Lactose [illegible] mg Hydroxypropyl cellulose [illegible] mg, [illegible] mg in total

DETL:
(3) Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride [illegible] mg Crystalline cellulose [illegible] mg Lactose 23.3 mg Hydroxypropyl cellulose 10 mg Magnesium stearate 5 mg [illegible] mg in total

CLPR:
1. An ampicillin ester of the formula ##STR1## wherein R.sub.1 represents a hydrogen atom, a methyl group or an aryl group, and R.sub.2 represents a hydrogen atom or may be taken together with R.sub.1 to form a divalent carbon chain residue, or a pharmaceutically acceptable acid addition salt thereof.

CLPR:
7. An antibacterial agent comprising an antibacterially effective amount of an Ampicillin ester of the formula ##STR3## wherein R.sub.1 represents a hydrogen atom, a methyl group or an aryl group, and R.sub.2 represents a hydrogen atom or may be taken together with R.sub.1 to form a divalent carbon chain residue, or a pharmaceutically acceptable acid addition salt thereof, in combination with a pharmaceutically acceptable carrier.

[illegible]:
[illegible] divalent [illegible], or a pharmaceutically acceptable acid addition salt thereof.

[illegible]:
4. A process for the treatment of bacterial infectious disease which comprises [illegible] pharmaceutically acceptable acid addition salt thereof.

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[illegible]

BSUB:
[illegible] .beta.-lactam [illegible] by enzymatic acylation of the parent amino .beta.-lactam with an acylating agent.

BSUB:
Today, semisynthetic .beta.-lactam derivatives such as ampicillin, amoxicillin, cefaclor, cephalexin, cephadroxil and cephaloglycin are, in an industrial scale, prepared by chemical methods, for example by reacting an amino .beta.-lactam such as 6-aminopenicillanic acid, usually having its carboxyl group protected, with an activated side chain derivative, followed by the removal of the protecting group by hydrolysis. For example, ampicillin (6-(D-.alpha.-amino phenylacetamido-penicillanic acid) can be prepared by [illegible] 6-APA, having a suitable protected carboxyl group, with [illegible], followed by removal of the protecting group by hydrolysis. [illegible] and organic solvents like methylene chloride and silylation reagents.

BSUB:
Within the last years, there has been an increasing amount of publications [illegible] enzymatic preparation of penicillins and [illegible] acylating agent, [illegible] .beta.-lactam [illegible], 6-APA or 7-ADCA. For example, enzymatic production of ampicillin from 6-APA and a D-phenyl-glycine derivative (such as a lower alkyl ester) is known from West German patent application having publication No. [illegible], Austrian Patent No. 243,986, Dutch patent application No. 70-09138, West German patent application having publication No. 2,[illegible], European patent application having publication No. 339,751 and [illegible]

BSUB:
[illegible]

BSUB:
[illegible]

[illegible]

BSPR:
It has now, surprisingly, been found that improved process conditions are obtained if the enzymatic acylation of the amine .beta.-lactam is performed at [illegible] concentrations of both the parent amino .beta.-lactam and the [illegible] acylating agent. Preferably the concentrations of the parent amino .beta.-lactam and [illegible] acylating agent are higher than [illegible], preferably higher than [illegible] of the lower value of both the saturated concentration of the parent amino .beta.-lactam and of the saturated concentration of the corresponding acylating agent.

DEPR:
The .beta.-lactam derivative formed may precipitate during the reaction and, e.g., the acid form of the acylating agent such as D-phenylglycine and D-p-hydroxyphenylglycine may precipitate. Hence, in some cases the reaction [illegible].

DEPR:
The (parent) amino .beta.-lactam has a free amino group which is acylated by the reaction according to this invention. The amino .beta.-lactam may conveniently be 6-APA, 7-ADCA, 7-ACA or 7-ACCC.

DEPR:
The amino .beta.-lactam, for example 6-APA or 7-ADCA, used in the process of this invention may be obtained by enzymatic hydrolysis of the fermented penicillins or cephalosporins, (for example penicillin V, penicillin G or cephalosporin C) or their ring enlarged analogues (for example V-DCA and G-DCA) or derivatives thereof followed by removal of the hydrolysis by-product, if desired (phenoxyacetic acid etc.). Advantageously, the crude solution can be used directly without further purification or dilution.

DEPR:
The acylating agent may be in an activated form. Preferably, the acylating agent is an amide or an ester. The acylating agent may be a derivative of D-phenylglycine, D-p-hydroxyphenylglycine, D-2,5-dihydrophenylglycine or mandelic acid, such as a lower alkyl ester (methyl, ethyl, n-propyl or isopropyl ester) or an amide which is unsubstituted or substituted in the --CONH.sub.2 group. The acylating agent may be used in the form of a salt, for example, the [illegible] salt or the H.sub.2 SO.sub.4 salt. The acylating agent may be added in an active form or the active form may be formed in situ.

DEPR:
The solubility of the acylating agent such as the D-phenylglycine or D-p-hydroxyphenylglycine derivative will vary with the identity of the derivative and with the composition of the reaction medium. In an aqueous system as used in the examples, the solubility of the hydrochloride salt of D-phenylglycine amide is typically approximately [illegible] mM. However, the solubility is very dependent on the salt components in the solution, as well as on the pH value and the temperature of the solution. As a further example, the solubility of the sulphate form of D-phenylglycin amide is about 3.3 M within a pH range from [illegible] to 6.5.

DEPR:
Examples of .beta.-lactam derivatives that may be prepared by the process of this invention are ampicillin, amoxicillin, cefaclor, cephalexin, cephadroxil, [illegible].

DEPR:
The enzyme [illegible] in the process of this invention may be any enzyme [illegible]. Such enzymes have been known since [illegible]. Enzymes [illegible] are, for example, [illegible] penicillin [illegible] or penicillin acylase and classified as E.C. 3.5.1.11. A number of microbial

enzymes are known to have this activity, derived from for example Acetobacter, Alcaligenes, Xanthomonas, Mycoplana, Protaminobacter, Aeromonas (West German patent application having publication No. 2,163,792) Pseudomonas (Austrian Patent No. [illegible]), Flavobacterium (Dutch patent application No. [illegible]), Aphanocladium, Cephalosporium (West German patent application having publication No. 2,621,618), Acetobacter pasteurianum, Alcaligenes faecalis, Bacillus megaterium, Xanthomonas citrii (European patent application having publication No. [illegible]), Kluyvera citrophila (Agr. Biol. Chem. [illegible]), [illegible] (West German patent application having publication No. [illegible]). [illegible] enzyme is commercially available. The enzyme used may be a so-called penicillin G acylase, cephalosporin C amidase. In this connection, reference is, inter alia, made to Hakko to Kogyo [illegible], 216 et seq., the content of which is incorporated by reference.

DEPR:
It is preferred to use the enzyme in a reusable form, for example, in [illegible] form. [illegible] may be done by any known method. [illegible] the enzyme is commercially available from Boehringer Mannheim GmbH, Germany, under the trade name Enzygel.RTM., and Recordati under the trade name Super Enzygel.RTM.. Preferably, as enzyme Penicillin G acylase, immobilized on a carrier consisting of a gelling polycarbohydrate and a polymer containing free amino groups, is applied.

DEPR:
The suitable pH value depends, inter alia, on the type of the enzyme used. Using Escherichia coli enzyme, the pH value is typically in the range from [illegible] to [illegible], preferably in the range from [illegible] to [illegible]. For the preparation of amoxicillin, a pH value in the range from [illegible] to [illegible] is preferred. Control of the pH value may be used.

DEPR:
Suitable enzyme concentrations may be from 1 to 100 U/ml (1 U = one unit of enzyme activity, see below).

DEPR:
Using the process according to this invention, an extraordinary high molar ratio between the amount of beta.-lactam derivative which can be recovered and the total amount of [illegible] acylating agent can be obtained. These high ratios are obtained using the teachings of this invention and properly controlling the concentration of the acylating agent, the ratio between the concentration of acylating agent and the starting amino beta.-lactam, the pH value and the enzyme. Thus, a ratio of 2.4 was obtained in Example 1 below using the process according to the present invention. In a comparative batch process, vide Example 2 below, a molar ratio of only 1.4 was obtained. In addition, the yields of isolated product obtained in this example were [illegible] and [illegible], respectively.

DEPR:
Herein, the following abbreviations have been used: 7-ADCA is 7-aminodesacetoxycephalosporanic acid, 7-ACA is [illegible], [illegible], 6-APA is 6-aminopenicillanic acid, D-HPG is D-p-hydroxyphenylglycine, D-PG is D-phenylglycine, D-PGA is D-phenylglycine amide, D-PGM is D-phenylglycine methyl ester, D-HPGM is D-p-hydroxyphenylglycine methyl ester, [illegible], D-HPGA is D-p-hydroxyphenylglycine amide, and [illegible] is [illegible].

DEPR:
As enzyme Penicillin G acylase [illegible], immobilized on a carrier consisting of a gelling polycarbohydrate and a polymer containing free amino groups according to European patent [illegible], is applied.

DETD:
As definition of penicillin G acylase activity the following is used: one unit (U) corresponds to the amount of enzyme that hydrolyses per minute 1 .mu.mole penicillin G under standard conditions ([illegible] g/l Penicillin G potassium salt, [illegible] M potassium phosphate buffer, pH value [illegible], [illegible].degree. C.).

DETD:
The equipment for this experiment consisted of (see FIG. 1) a thermostated reactor having a volume of 1.5 liters, equipped with a three-bladed impeller and a sieve with [illegible] openings at the bottom. The reactor was connected to an autotitrator system using 6 M sulphuric acid as titrant. A valve was positioned at the outlet of the reactor. The outlet of the valve was connected via a pump to a basket centrifuge equipped with a polypropylene bag having a porosity of 1-5 .mu.m. The outlet from the centrifuge was connected via a pump to a feed tank equipped with a stirrer and a glass sinter bottom. The outlet from the feed tank was connected via a pump to the reactor.

DETD:
A mixture consisting of D-HPGM ([illegible] g, [illegible] mmol) and 6-APA ([illegible] g, [illegible] mmol) in [illegible] ml water was added to the reactor with the bottom valve closed. The stirring was started. Immobilized Penicillin G acylase ([illegible] U, size [illegible] .mu.m) made up to [illegible] ml was added to the reactor. The pH value was maintained at 6.1. The reaction temperature was about 20.degree. C. Under these conditions, the reaction mixture was almost saturated with D-HPGM and 6-APA. Then, the bottom valve was opened allowing the reaction mixture from the reactor to enter the centrifuge. Thereafter, the mother liquor from the centrifuge was pumped into the feed tank wherein D-HPGM (21.7 g, 120 mmol) and 6-APA ([illegible] g, [illegible] mmol) were loaded. The total volume of the suspension in the feed tank was kept at about 75 ml. A flow of about 100 ml/min was maintained in the system. The concentration of reaction components in the reactor, in the reactor outlet, in the centrifuge outlet, in the feed tank and in the feed tank outlet were monitored by analytical HPLC.

DETD:
As the reaction proceeded, the amoxicillin and D-HPG formed started to precipitate out of solution. The crystals were separated from the immobilized enzyme particles by the bottom sieve in the reactor and the crystal suspension was [illegible] the centrifuge where the crystals were separated from the mother liquor. [illegible], which was now unsaturated with respect to D-HPGM and 6-APA, passed through the feed tank, some of the solid D-HPGM and 6-APA present dissolved such that the outlet from the feed tank contained saturated D-HPGM and 6-APA. When the total concentrations of D-HPGM and 6-APA came down to about 225 mM HPGM and 225 mM 6-APA, more solid substrate was added to the feed tank. At intervals the crystals in the centrifuge were washed with water, the washing liquid was added to the reactor. The amount of water used was sufficient to keep the volume in the reactor at its starting [illegible] crystals in the centrifuge, the centrifuge was [illegible].

DETD:
After about 12 hours, the dosing of D-HPGM and 6-APA was stopped. After 14 hours, the 6-APA concentration reached [illegible] mM and the reaction was stopped. The amounts of reaction components are given in Table 1, below.

DETD:
A comparative experiment was performed at batchwise conditions and the reaction was stopped at the moment where the optimum yield of amoxicillin was [illegible]. The reaction temperature was about [illegible].degree. C., the pH value was [illegible] units of the enzyme, mentioned in this example above, was used. The total volume of the reaction mixture was 1 liter. The reaction was performed in the reactor mentioned above. After [illegible] hours the [illegible] valve was opened, and the crystals were separated from the immobilized enzyme particles by the bottom sieve in the reactor. The crystal suspension was filtered. The amounts of reaction components are given in Table 2, below.

[illegible]:
A mixture consisting of D-HPGA ([illegible] g, [illegible] mmol) and 6-APA in [illegible] ml water, which was adjusted at a pH value of [illegible] by adding 4 M ammonium hydroxide, was added to the reactor with the bottom valve closed. The stirring was started. Immobilized Penicillin G acylase (Assem. [illegible], size [illegible] .mu.m) made up to [illegible] ml was added to the reactor. The pH value was maintained at 6.[illegible]. The reaction [illegible] [illegible] these conditions the reaction [illegible] with D-HPGA and 6-APA. Then the bottom valve was [illegible] [illegible], the mother liquor [illegible] centrifuge was pumped into the feed tank [illegible] D-HPGA [illegible] g, [illegible] mmol) and 6-APA ([illegible] g, [illegible] mmol) were [illegible]. The total volume of the suspension in the feed tank was kept at about [illegible] ml. A flow of about [illegible] ml min was maintained in the system. The concentration of reaction components in the reactor, in the reactor outlet, in the centrifuge outlet, in the feed tank, and in the feed tank outlet were monitored by analytical HPLC.

[illegible]:
The crystals formed in the reactor were separated from the immobilized enzyme particles by the bottom sieve in the reactor. The crystal suspension was fed to the centrifuge where the crystals were separated from the mother liquor. The mother liquor was passed through the feed tank. When the total concentrations in the feed tank came down to about 150 mM HPGA and 225 mM 6-APA, more solid was added to the feed tank. At intervals the crystals in the centrifuge were washed with water, the washing liquid was added to the reactor. The amount of water used was sufficient to keep the volume in the reactor at its starting level. After washing the crystals in the centrifuge, the centrifuge was emptied.

DEPR:
After about 12 hours, the dosing of 6-APA to the feed tank was stopped while the D-HPGA dosing was maintained in order to keep D-HPGA at saturation. After 14 hours, the 6-APA concentration reached 20 mM and the reaction was stopped. The amounts of reaction components are given in Table 3, below.

[illegible]:
[illegible]

[illegible]:
HPLC Analysis of Ampicillin and Cephalexin

DEPL:
Enzymatic Preparation of Amoxicillin from D-HPGM and 6-APA

DEPL:
[illegible] Preparation of Amoxycillin from D-HPGA and 6-APA

[illegible]:
[illegible] times in minutes: [illegible] D-PG; [illegible] 7-ADCA; [illegible] 6-APA; [illegible] D-PGM; 13.4 Cephalexin; 13.[illegible] Ampicillin; 1[illegible] D-PGM.

DEPV:
Retention times in minutes: 1.[illegible] D-p-hydroxyphenylglycine; [illegible] D-HPGM; [illegible] D-HPGA; [illegible] 6-APA; [illegible] Amoxicillin.

[illegible]:
TABLE [illegible] [illegible] crystal cake in [illegible]
[illegible] 6-APA [illegible] Amoxicillin. [illegible] D-HP[illegible] [illegible] mmol [illegible] mmol
Based on the above results, the [illegible] yield [illegible] calculated amoxicillin yield (isolated product, based on 6-APA added): [illegible]. Amoxicillin productivity: [illegible] mmol hour liter.

DETL:
TABLE [illegible] substrate crystal [illegible] in
solution added at the end at the end
D-HPGM [illegible] mmol [illegible] mmol [illegible] mmol 6-APA [illegible] g [illegible] mmol [illegible] mmol 14 mmol Amoxicillin. 3H.sub.2 O [illegible] g [illegible] mmol [illegible] mmol D-HPG [illegible] mmol [illegible] mmol
Amoxicillin yield [illegible] (based on 6-APA [illegible]). Amoxicillin productivity: [illegible] mmol/hour/liter. [illegible]

DETL:
TABLE 3 substrate crystal at in
solution added the end at the end
D-HPGM 443.[illegible] g 1.[illegible] 22.4 [illegible] 2317 mmol [illegible] mmol 155 mmol 6-APA 443.[illegible] g [illegible] mmol [illegible] mmol [illegible] mmol Amoxicillin. 3H.sub.2 O [illegible] g [illegible] mmol [illegible] mmol D-HPG [illegible] mmol [illegible] mmol
Amoxicillin yield [illegible] (based on 6-APA [illegible]). Amoxicillin productivity: 1[illegible] mmol/hour/liter. [illegible]

CLPR:
14. The process according to claim 1, wherein the amino .beta.-lactam is selected from the group consisting of 6-aminopenicillanic acid, 7-amino-desacetoxycephalosporanic acid, 7-aminocephalosporanic acid, 7-amino-3-chloro-3-cephem-4-carboxylate and 7-amino-3-3-cephem-4-carboxylate.

CLPR:
15. The process according to claim 1, wherein the acylating agent is selected from the group consisting of an activated form of D phenylglycine, D p-hydroxyphenylglycine, D-2,5-dihydrophenylglycine and mandelic acid.

CLPR:
16. The process according claim 1, wherein the .beta.-lactam compound is selected from the group consisting of ampicillin, amoxicillin, cefaclor, cephalexin, cephadroxil, cephradine, epicillin and cefamandol.

WEST

End of Result Set

Generate Collection

[illegible] Entry [illegible] File: DWPI [illegible]

DERWENT-ACC-NO: [illegible]
DERWENT-WEEK: [illegible]

TITLE: Recovery of D-phenyl-glycine amide from antibiotic [illegible] - by formation of pure, easily sepd. recyclable Schiff base, used in enzymatic prepn. of cephalexin, cefaclor, ampicillin, etc.

INVENTOR: [illegible]

PATENT-ASSIGNEE:

ASSIGNEE	CODE
DSM NV	STAM

PRIORITY-DATA:

1993BE-[illegible]	July 19, 1993

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
ES 2110251 T3	February 1, 1998	N/A	000	C12P035/04
WO 9503420 A1	February 2, 1995	E	012	C12P035/04
BE 1007296 A3	May 9, 1995	F	000	C12P000/00
EP 712443 A1	May 22, 1996	E	000	C12P035/04
[illegible]	July [illegible]	N/A	[illegible]	C12P035/04
[illegible]	[illegible]	F	[illegible]	[illegible]
[illegible]	[illegible]	N/A		[illegible]

DESIGNATED-STATES: CN JP KR US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE AT DE ES FR GB IT NL PT AT DE ES FR GB IT NL PT

CITED-DOCUMENTS: EP 44[illegible]; EP 44[illegible]; US 4[illegible]; [illegible]

PUB-NO	APPL-[illegible]	APPL-NO	APPL-[illegible]
[illegible]	July 1[illegible], 1994	1994[illegible]	N/A
[illegible]	N/A	EP 71244[illegible]	Based on
WO [illegible]1342[illegible]A1	July 13, 1994	1994WO-NL00161	N/A
BE 1007296A3	July 13, 1993	1993BE-0000750	N/A
EP 712443A1	July 13, 1994	1994EP-0924401	N/A
[illegible]	July 1[illegible], 1994	1994W[illegible]-NL[illegible]	N/A
[illegible]	N/A	W[illegible]	Based on
[illegible]	July 1[illegible], 1994	[illegible]	N/A
EP 712443B1	July 1[illegible], 1994	1994EP-0924401	N/A
EP 712443B1	July 1[illegible], 1994	1994WO-NL00161	N/A
EP 712443B1	N/A	WO 9[illegible]34[illegible]	Based on
DE 694[illegible]E	July 1[illegible], 1994	1994DE-[illegible]	N/A
DE 694[illegible]E	July 1[illegible], 1994	1994EP-0924401	N/A
DE 694[illegible]E	July 1[illegible], 1994	1994WO-NL00161	N/A
DE 694[illegible]E	N/A	EP 71244[illegible]	Based on
DE 694[illegible]E	N/A	WO 9[illegible]34[illegible]	Based on

INT-CL (IPC): C07C 231/20; C12P 1/00; C12P 17/10; C12P 35/04; C12P 37/04

ABSTRACTED-PUB-NO: EP 712443B
BASIC-ABSTRACT:

Prepn. of a beta-lactam deriv. is claimed in which a beta-lactam nucleus is coupled to D-phenylglycine amide in an enzymatic reaction, and the enzyme, solid D-phenylglycine, and beta-lactam deriv are sepd out. After the enzymatic reaction, the prtd. mixt. (from which at least the enzyme and solid D-phenylglycine have been removed), is treated with an aldehyde at a pH of [illegible] and the Schiff base of D-phenylglycine amide is sepd. out.

USE - The coupling reaction is used for prepn of important antibiotics, including ampicillin, cephalexin and cefaclor, in which the beta-lactam nuclei are respectively e.g. 6-aminopenicillanic acid (6-APA), 7-amino-desacetoxy-cephalosporanic acid (7-ADCA), and 7-amino-3-chloro-3-cephem-4-carboxylate; also [illegible] with 7-ACA, and related cpds.

ADVANTAGE - The D-phenylglycine amide deriv is easily sepd. out in pure form, pref before the beta-lactam prod as the solubility of the latter is higher; recovery and purifcn of this prod by known methods, is simplified. Recovery and recycle of the D-phenylglycine amide deriv is by simple filtration or extn. If, in particular, the aldehyde benzaldehyde is used, excess aldehyde can serve as extn solvent, although other solvents, or mixts with PhCHO, can be used. The Schiff base is then split with acid, eg H_2SO_4, and recycled. As an excess of amide is used in the coupling to obtain a high yield of beta-lactam prod, recovery of the excess is necessary in order to provide a commercially attractive process; the reaction mixt typically contains 1-[illegible] moles of D-phenylglycine amide, [illegible] equivs of D-phenylglycine and [illegible] equivs of [illegible], all per mole of [illegible] prod.
ABSTRACTED-PUB-NO:

W[illegible]A
EQUIVALENT-ABSTRACTS:

[illegible]

[illegible]

[illegible]

[illegible] H2SO4 [illegible]

[illegible]

TITLE-TERMS: [illegible] PHENYL GLYCINE AMIDE ANTIBIOTIC [illegible] FORMATION [illegible] [illegible] [illegible] AMPICILLIN

[illegible]

CHEMICAL-CODES:

Chemical Indexing M[illegible]

Fragmentation Code

[illegible]

Specific Compounds

[illegible]

Registry Numbers

[illegible]

Chemical Indexing M[illegible]

Fragmentation Code

[illegible]

Specific Compounds

[illegible]

Registry Numbers

[illegible]

Chemical Indexing M[illegible]

Fragmentation Code

[illegible]

Specific Compounds

UNLINKED-DERWENT-REGISTRY-NUMBERS: 0289P; 1986P

SECONDARY-ACC-NO :
CPI Secondary Accession Numbers: [illegible]

WEST

Generate Collection

L4: Entry 4 of 11 File: USPT [illegible]

DOCUMENT-IDENTIFIER: US [illegible] A
TITLE: Penicillin and cephalosporin intermediates

ABPL:
Novel penicillin and cephalosporin intermediates for the synthesis of 6-APA or 7-aminocephem compounds involving formation of a 6- or 7-phosphite amide compound and the conversion of such compound to a desired 6-APA or 7-aminocephem compound.

BSPR:
Numerous methods for the synthesis of penicillins or cephalosporins have been described in the literature. The major part of these syntheses involves the reaction of 6-aminopenicillanic acid (in the following referred to as 6-APA), salts and esters thereof or the corresponding 7-aminocephem compounds with an activated derivative of the acid, with which the amino group is to be acylated.

BSPR:
In one aspect of the invention there is provided a novel intermediate for the synthesis of derivatives of 6-APA or 7-aminocephem compounds in high yields.

DEPR:
The structure of the compounds having formula I has been established on the basis of their IR and NMR spectra ([illegible]). The spin-spin coupling pattern of the latter shows that the 6.alpha.-hydrogen of the 6-APA derivative or the corresponding 7.alpha.-hydrogen of the 7-aminocephem derivative is strongly influenced by the introduction of the phosphorous atom.

DEPR:
In 6-APA derivatives with a free NH.sub.2 -group the proton in the 6-position is normally seen as a doublet at .delta.=[illegible] ppm ([illegible]). However, in an example of a 6-APA compound having the formula I, cf. Example 1, this proton is found as a multiplet consisting of [illegible] peaks in the region of .delta.=[illegible] ppm ([illegible]), from which by first order analysis the following coupling constants can be obtained:

BSPR:
It is even more surprising that a compound having the formula I, wherein R is a secondary ammonium group can be reacted with a halophosphite compound to form the corresponding phosphiteamide compound. However, it has been found that for example 6-aminopenicillanic acid or 7-aminodesacetoxycephalosporanic acid, when [illegible] in the presence of two [illegible] diethylamine reacts with for example ethylene chlorophosphite so as [illegible] the diethylammonium salt of
6-ethylenephosphiteamidopenicillanic acid or
7-ethylenephosphiteamidodesacetoxycephalosporanic acid and diethylammonium chloride. It might have been expected that ethylene chlorophosphite would react exclusively with the excessive mole of secondary amine.

BSPR:
[illegible] in the preparation of penicillin and cephalosporin compounds from 6-aminopenicillanic acid, 7-aminocephem acids or [illegible], an acid halide is frequently used in the presence of a

[illegible]

[illegible]:
[illegible] 6-APA [illegible] 6-APA. [illegible] further acylation reaction it may be preferred [illegible] the excessive alkyl amine but, on the other hand, a hydrohalide [illegible] is necessary to promote the reaction.

[illegible]:
[illegible] triethylamine [illegible] at this stage of the synthesis, 1 mole of trialkylsilylhalide, 1 mole of [illegible] chlorophosphite or 1 mole of a reactive alkyl halide may be added to form the corresponding ester.

BSPR:
Among other activated acid derivatives which have been used in the acylation of the amino group of 6-APA or derivatives thereof are various forms of [illegible] esters, e.g. p-nitrophenyl, cyanomethyl esters and the like.

BSPR:
Few syntheses of penicillin or cephalosporin derivatives are known in which a free carboxylic acid is reacted with a derivative of 6-APA or a 7-aminocephem compound.

BSPR:
The specification of British Patent No. 1.268.536 discloses that 6-isocyanato penicillanic acids may be prepared by reacting phosgene with a 6-APA ester. The 6-isocyanato penicillanic ester may then be reacted with a free acid to yield a penicillin derivative. However, this reaction can only be performed with moderate yield, and furthermore this known synthesis is not fully acceptable because of the use of phosgene which is poisonous and technically difficult to handle.

BSPR:
The above reaction can be illustrated by the following reaction scheme, in which a 6-APA derivative of the formula I is reacted with a carboxylic acid: ##STR6## in which R has the meaning defined above, and R.sup.3 is an organic group.

BSPR:
In a further embodiment of the method of the invention phthalide esters of [illegible] can be prepared in high yields from [illegible] acids without [illegible] epimerization [illegible].

BSPR:
[illegible] millimoles of 6-aminopenicillanic acid (6-APA) are dissolved in [illegible] ml [illegible] by addition of [illegible] millimoles of triethylamine, [illegible] To this solution is added in [illegible] After stirring [illegible] hour, during which time the temperature of the reaction mixture raised to room temperature, the triethylammonium chloride formed [illegible] millimoles is filtered off.

BSPR:
[illegible] millimoles of 6-aminopenicillanic acid are dissolved in [illegible] ml of [illegible] by addition [illegible] millimoles of triethylamine. The [illegible] millimoles of [illegible] is added in [illegible] under dry nitrogen. The mixture is

[illegible] -[illegible].degree. [illegible] [illegible] minutes.

BSPR:
Dry ether is added to the mixture to precipitate the triethylammoniumchloride formed. The salt is filtered off. The filtrate is evaporated to a white wax which is very hygroscopic. The product is stable in cold, dry solution and when stored under dry nitrogen. The product may contain small amounts of unreacted 6-aminopenicillanic acid, triethylammonium salt.

BSPR:
[illegible] g ([illegible] millimoles) of 6-aminopenicillanic acid are dissolved in [illegible] ml [illegible] by the addition of 1.4 ml ([illegible] millimoles) of triethylamine. The [illegible] -[illegible].degree. [illegible] and while stirring [illegible] ml ([illegible] millimoles) of 4-[illegible]-1,3,2-dioxaphospholan dissolved in [illegible] ml chloroform are added under dry [illegible] and stirring is continued for [illegible] minutes at -[illegible].degree. [illegible] and subsequently [illegible] minutes at room temperature. [illegible] ml dry benzene are added. The triethylammoniumchloride formed is separated by filtration. During said filtration part of the product is lost, but the filtration is normally unnecessary when the product is to be used for further reactions.

BSPR:
[illegible] g ([illegible] millimoles) of 6-aminopenicillanic acid are dissolved in [illegible] ml dry acetonitrile by the addition of 1.4[illegible] ml ([illegible] millimoles) of triethylamine while stirring. 1.4[illegible] ml ([illegible] millimoles) of 2-chloro-1,3,2-thiaoxaphospholan in 2 ml dry acetonitrile are added dropwise under dry nitrogen and at a temperature of about -40.degree. C. Subsequently the solution is stirred for about 1 hour at room temperature.

BSPR:
1.03 ml (10 millimoles) of diethylamine are added to 1.08 g (5 millimoles) of 6-aminopenicillanic acid suspended in 10 ml dry acetonitrile. After stirring in a nitrogen atmosphere for 2 minutes, a clear solution is obtained.

BSPR:
To a suspension of [illegible] g ([illegible] millimoles) of 6-APA in 10 ml of dry methylene chloride are added 1.4 ml ([illegible] millimoles) of triethylamine, and the mixture is stirred at room temperature until a clear solution is formed. The solution is cooled to -40.degree. C, and [illegible].48 ml ([illegible] millimoles) of ethylenechlorophosphite dissolved in [illegible] ml of dry methylenechloride is added under dry nitrogen. The mixture is stirred for [illegible] minutes at -40.degree. C, 15 minutes at 0.degree. C and [illegible] minutes at room temperature, thereafter [illegible] ml ([illegible].3 millimoles) of trimethylchlorosilane dissolved in [illegible] ml of methylenechloride is added. The mixture is stirred for [illegible] minutes at room temperature, and subsequently the precipitated triethylamine hydrochloride is filtered off. The solution is evaporated to form a hard, pale yellow oil.

BSPR:
[illegible] g of 6-APA is suspended in 15 ml of dry methylene chloride under nitrogen. To this mixture are added 1.42 ml of dry triethylamine and [illegible].62 ml of trimethylchlorosilane. After stirring for 1 hour the reaction mixture is cooled to -[illegible].degree. C, and a solution of [illegible] ml of [illegible]-phenylene-chlorophosphite in [illegible] ml of dry methylene chloride is added [illegible]. When the addition is complete [illegible], the mixture is [illegible] minutes, and the solvent is removed in vacuo. Dry benzene is added, and the triethylammonium chloride formed is removed by filtration [illegible]. The benzene is removed in vacuum [illegible] of the [illegible] product [illegible] an amorphous substance.

BSPR:
[illegible] g ([illegible] millimoles) of D-(-)-.beta.-phenylglycylchloride, HCl are then [illegible] reaction mixture, and the mixture is stirred for [illegible] hours at -[illegible].degree. C. Subsequently [illegible] ml water and [illegible] are added and the mixture is stirred for [illegible] minutes at [illegible].degree.-[illegible].degree. C. The pH is adjusted [illegible] reaction mixture is filtered [illegible]

[illegible] which is thoroughly washed with water. The [illegible] phase is separated and the aqueous phase is washed twice with [illegible] ml methylene chloride. The volume of the aqueous phase is reduced to [illegible] ml under vacuum and pH [illegible] with [illegible] hydrochloric acid. [illegible] g ([illegible] millimoles) of .beta.-naphthol dissolved in [illegible] ml ethanol are added during a period of [illegible] hours while stirring. Stirring is continued for about [illegible] hours while lowering the temperature to [illegible].degree. C. Finally the reaction mixture is left to stand overnight at [illegible].degree. C. The precipitated cephalexin-.beta.-naphthol complex is separated by filtration and washed with water and butylacetate. The washed precipitate is suspended in [illegible] ml water and [illegible] ml butylacetate and the pH is adjusted at [illegible] with a [illegible] [illegible] solution. Subsequently the mixture is filtered and the aqueous phase is washed twice with [illegible] ml butylacetate. The pH-value of the aqueous phase is adjusted at 4.[illegible] with triethylamine and the volume of the mixture is reduced under vacuum to [illegible] ml. [illegible] ml of [illegible]-dimethoxyethane are added and the mixture is stirred while being cooled to [illegible].degree. C over a period of about [illegible] hours. The mixture is left to stand overnight in a refrigerator at [illegible].degree. C. The precipitate formed is separated by filtration, washed with water and dried in an exsiccator.

BSPR:
4.33 g (21 millimoles) of D(-)-phenylglycylchloride, hydrochloride are added in portions over a period of 1 minute to a solution in methylene chloride of [illegible] millimoles of trimethylsilyl-6-(ethylenephosphiteamido)penicillanate prepared as described in Example [illegible] and without removing triethylammonium chloride, at a temperature of [illegible].degree. C. The progress of the reaction is followed by penicillinase titration which after a period of [illegible] hours at [illegible].degree. C shows that a yield of above 70[illegible] has been obtained. At this time the reaction mixture is poured into 100 ml ice-water, and after stirring for 15 minutes the reaction mixture is filtered. The water phase is covered by 20 ml ethylacetate and the pH-value of the water phase is adjusted at 2 with a NaOH solution. Subsequently, the ampicillin formed is precipitated as a sparingly soluble salt with .beta.-naphthalenesulphonic acid while maintaining the pH-value at 2. The reaction mixture is left to stand for 1[illegible] hours at [illegible].degree. C and is subsequently filtered and the residue is washed with [illegible] N HCl and ethylacetate. After drying in vacuum [illegible] g white ampicillin .beta.-naphthalenesulphonic acid salt corresponding to 64.[illegible] of the theoretical amount are obtained. A high-voltage electrophogram showed one spot with mobility identical to that of authentic ampicillin. The IR spectrum was identical to that of the .beta.-naphthalenesulphonic acid salt of authentic ampicillin.

BSPR:
[illegible] g ([illegible] millimoles) of 6-aminopenicillanic acid are dissolved in [illegible] ml dry alcohol-free chloroform by the addition of [illegible] ml ([illegible] millimoles) of triethylamine, [illegible] ml ([illegible] millimoles) of trimethylchlorosilan are added dropwise and the reaction mixture is stirred for 1 hour at room temperature. At [illegible].degree. C [illegible] ml ([illegible] millimoles) of ethylene chlorophosphite dissolved in 2 ml dry alcohol-free chloroform are added dropwise. The reaction mixture is stirred for 1 hour at [illegible].degree. C.

BSPR:
[illegible] g of [illegible] ([illegible] millimoles) and [illegible] g of pyridine hydrochloride are dissolved in [illegible] ml of dry methylene chloride and heated to reflux. A [illegible] ([illegible] millimoles) of trimethylsilyl [illegible]-ethylenephosphiteamido)penicillanate in [illegible] ml of dry methylene chloride is added during [illegible] minutes. Enzymatic titration [illegible] minutes after the addition is completed shows a penicillin yield of [illegible], while titration after one and two hours both shows [illegible] yield.

BSPR:
[illegible] millimoles of N-tert.-butoxycarbonyl-D-(-)-.alpha.-phenylglycine trimethylsilylester prepared from [illegible] g ([illegible] millimoles) of N-tert.-butoxycarbonyl-D-(-)-.alpha.-phenylglycine, [illegible] ml of chloroform, [illegible] ml ([illegible] millimoles) of triethylamine and [illegible] ml ([illegible] millimoles) of trimethylchlorosilan by stirring for [illegible] minutes are added to a solution of [illegible]

[illegible]
[illegible] toluene are added, the [illegible] is evaporated, and the mixture is refluxed [illegible] .degree. C. for [illegible] hours. Then the mixture is evaporated in vacuum to dryness. The residue is dissolved in methylene chloride and washed twice with diluted hydrochloric acid and twice with a solution of sodium hydrogen [illegible] and twice with a solution of sodium hydrogen carbonate. The [illegible] is dried over Na.sub.2 SO.sub.4 and evaporated in vacuum to form an amorphous powder. Yield: [illegible]
[illegible]

[illegible]:
To a solution of [illegible] millimoles of [illegible]-penicillanate (prepared as described in Example [illegible], from [illegible] of 6-APA) in dry methylene chloride are added [illegible] ([illegible] millimoles) of phenoxyacetic acid, and the mixture is stirred at room temperature with dry air bubbling through. After a reaction period of [illegible] hours at room temperature the reaction mixture shows a penicillin yield of [illegible] by [illegible] titration. The reaction mixture is cooled to [illegible].degree. C, and [illegible] ml of pyridine are added followed by [illegible] ml of dimethylsulfoxide, whereafter the reaction mixture is poured into [illegible] ml of ice-cold [illegible] NaCl solution and stirred for 30 minutes. Then 150 ml of ethylacetate are added and the pH adjusted to 2. After 30 minutes the phases are separated, and the water phase is extracted three times with ethylacetate. 25 ml of water are added to the combined organic phases, and the pH is adjusted to 7 with KOH. The water phase is separated, 150 ml of n-butanol added and the water removed by azeotropic vacuum destillation. The crystalline crude product thus precipitated is filtered off. Yield: 3.16 g (81%) of a purity of 76% determined by penicillinase titration. The white, crystalline product shows a characteristic absorption in the IR spectrum corresponding to that of the potassium salt of phenoxymethylpenicillin, and the NMR spectrum also shows signals which are characteristic of said compound.

BSPR:
[illegible] g ([illegible] millimoles) of phenoxyacetic acid is added to a solution of [illegible]',[illegible]',[illegible]'-trichloroethyl-3-methyl-7.beta.-(ethylenephosphiteamido)-ceph-3-em-4-carboxylate in acetonitrile prepared as described in Example [illegible], except for the filtration to remove the precipitate of triethylammonium chloride. The mixture is stirred for [illegible] hours at room temperature. The reaction mixture is then poured into ice-water and extracted three times with ethylacetate. The combined organic phases are washed three times with [illegible] N sulfuric acid and three times with saturated sodium hydrogen carbonate solution, dried over MgSO.sub.4 and evaporated in vacuum to form an amorphous powder:

BSPR:
4.7[illegible] g of N-carbobenzoxy-D-[illegible]-phenylglycine in dry methylene chloride at room temperature are added to the product ([illegible] g of benzyl [illegible]-ethylenephosphiteamidopenicillanate) of the reaction described in Example [illegible] under nitrogen. The mixture is stirred for 4 hours, evaporated to dryness, [illegible] ethylacetate and shaken with ice-water (twice), [illegible] (twice) and finally with ice-water (twice). The solution is dried [illegible] and evaporated in vacuum to give [illegible] g [illegible] amorphous [illegible].

[illegible]:
[illegible] g ([illegible] millimoles) of N-(tert-butoxycarbonyl)-D-(-)-.alpha.-phenylglycine are added to a solution of [illegible]',[illegible]',[illegible]'-trichloroethyl-3-methyl-7.beta.-(ethylenephosphiteamido)-ceph-3-em-4-carboxylate in acetonitrile, prepared as described in Example [illegible], except for the filtration to remove the precipitated triethylammonium chloride. The mixture is stirred for [illegible] hours. Then the reaction mixture is poured into ice-water and extracted three times with ethylacetate. The combined organic

[illegible]

[illegible]:
[illegible] g of 6-aminopenicillanic acid are dissolved in [illegible] ml of acetonitrile by addition of [illegible] ml of triethylamine, and the solution formed is cooled to [illegible]

[illegible]:
A solution of the triethylammonium salt of 6-ethylene phosphite amidopenicillanic acid was prepared from 2.16 g (10 millimoles) of 6-aminopenicillanic acid in the manner described in Example 1 and the triethylammonium chloride formed was removed by filtration.

BSPR:
The NMR spectrum ([illegible]H.sub.3 CN) of a sample of the reaction solution showed that all 6-aminopenicillanic acid had been converted into the corresponding [illegible] compound, and the following characteristic signals were obtained:

BSPL:
has the meaning defined above with a salt of 6-APA or of 7-aminocephem acid or a derivative of said acids.

BSPL:
The coupling constants J.sub.P-N-C-H, J.sub.H-N-C-H and J.sub.H-C-C-H are consistent with those found in literature (for P-N-CH.sub.3 compounds are normally found J.sub.P-H [illegible] 4.5 - 25 Hz (Jackman and Sternhell: Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, vol. 5, p. 352 (1969))). The coupling J.sub.H-C-C-H = 4.5 Hz is also found in the signal (doublet) from the proton in the 5-position in 6-APA. This pattern in conjunction with the signal of the 3-hydrogen and the infrared absorption frequencies of the carbonyl group in the 3-position which are consistent with those normally found in 6-APA derivatives, is a conclusive proof for the presence of the structural element: ##STR5##

[illegible]:
[illegible]

WEST

Generate Collection

[illegible]

DOCUMENT-IDENTIFIER: US [illegible] A
TITLE: Process for preparing cephalosporanic acid compounds

BSPR:
[illegible] cephalosporanic acid derivatives by acylating 6-APA or 7-A[illegible]A and their derivatives with the hydrochloride of a-phenylglycine-chloride and derivatives thereof having a substituted phenyl group, whereby the said acid choride is obtained by reacting the substituted phenylglycine with reagents like phosphorus pentachloride, thionyl chloride and phosgene. Although improved processes for the preparation of D-(-)-2-(p-hydroxyphenyl)-glycyl-chloride hydrochloride and the crystalline hemidioxane solvate thereof are known from British Pat. No. 1,466,637 and No. 1,460,915 the acylation of 6-APA or 7-A[illegible]CA or its 3-methyl modification with the above-mentioned acylating agent did not hitherto lead to results aimed at, mainly because either the product formed was too impure that further recovery of a product of the required quality hardly appeared to be possible, or the starting D-2-(p-hydroxyphenyl)-glycyl chloride hydrochloride of the required quality (purity) is only available for economically unattractive prices, if at all.

BSPR:
A process for the preparation of amoxicillintrihydrate is also known from published German patent application DT 2611286 comprising the acylation of 6-APA, which has been previously silylated, with D-(-)-2-(p-hydroxyphenyl)glycylchloride hydrochloride and leading to yields, which seem to approach to some extent the present practical requirements.

BSPR:
However, in the preparation of D-(-)-2-(p-hydroxyphenyl)-glycyl chloride hydrochloride according to the British Pat. No. 1,460,915 and British Pat. No. 1,466,637, phosgene is used in a relatively difficultly manageable process in which a solid is reacted with a gas. Such a process is extremely expensive in a number of countries with very stringent safety regulations, if indeed it may be applied at all. For the same reason, the process described in British Pat. Nos. [illegible] and 1,341,[illegible] disclosing the preparation of 6-isocyanatopenicillanic acid and 7-isocyanatocephalosporanic acid derivatives from esters of 6-APA or 7-ACA or its 3-methyl modifications with phosgene and [illegible] penicillins or cephalosporins are left out of [illegible]

BSPR:
Less attractive features of this process are that the process is carried out [illegible], that solvents become mixed so that recovery thereof [illegible] more difficult, and that, when adding the dimethylacetamide [illegible], 6-APA, 7-ACA or 7-A[illegible] [illegible]

BSPR:
Furthermore, a number of patent applications and patents disclose preparation methods of .alpha.-aminoacyl-penicillanic acid derivatives by acylating 6-APA with mixed anhydrides derived from modified Dane salts of D-2-amino- p-hydroxyphenl -acetic acid, such as those described in German

[illegible] However, the yields resulting [illegible] purposes of the present invention, and moreover, the [illegible] available in economically unattractive quantities, if at all available.

[illegible]:
[illegible] further discloses the preparation of [illegible] 6-APA, 7-ACA [illegible] derivatives and these [illegible] thereof should, in a number of cases, lead to improved yields, as appears, such as from British Pat. No. 1,[illegible] disclosing the preparation of intermediate organosilane penicillins by reaction of 6-APA and those bi-functional silicon compounds. The organosilane derivatives are acylated [illegible] ampicillin for example, so that an expert from the contents of this [illegible] the use of the organosilane penicillins described [illegible] interesting yields in the preparation of amoxicillin. [illegible] not be confirmed by initial experiments.

BSPR:
Although it is further known from a number of patent applications such as Japanese Patent application No. 49-11468? and No. 49-048892, British patents No. 1,367,342 and No. 1,382,255 and German patent applications Ser. No. 2,460,649 and No. 2,621,618, to prepare amoxicillin from 6-APA and p-hydroxy-phenylglycine or lower alkyl esters thereof by enzymatic acylation, the processes of this type are also unsatisfactory for the deemed purpose in view of the yields obtained and or the presence of the acylating enzyme in the amoxicillin-containing solution obtained.

BSPR:
It has been found that the way in which the silylation is carried out is very important for the final yield, and the silylation is preferably carried out in dry methylene chloride containing 2 to 3 equivalents of a tertiary amine such as triethylamine and an equivalent amount of TMCS (about 2 equivalents for amoxicillin and cefadroxil and 3 equivalents for cefatrizine), in such a way that the signal [illegible] by a pH electrode is adjusted at the end of the [illegible] value [illegible], for example a pH scale value between [illegible] and [illegible], preferably [illegible] and [illegible], of a Radiometer pH meter type TTT1,? and a Radiometer [illegible] electrode or an Ingold, so-called cold electrode, at a temperature between [illegible].degree. and [illegible].degree. C. Therefore, disilylation of e.g. of 6-APA or 7-ADCA is preferably carried out with practically balanced mutual amounts of tri(lower alkyl) halosilane, such as TMCS, and tertiary amine (such as TEA).

[illegible]:
[illegible] the solution of the anhydride as prepared [illegible] a temperature of -[illegible].degree. C. or lower and a cooled solution of silylated 6-APA, 7-ACA or [illegible] derivatives thereof are added rapidly with stirring as well as possible so that a temperature of -15.degree. C. to -[illegible].degree. C. is reached, whereafter the reaction mixture is stirred for a further [illegible] to 3 hours. An excess of the formula V compound is preferably employed, the excess being dependent on the nature of the substituent of the 3-methyl group of the cephalosporanic nucleus. For the preparation of amoxicillin and cefadroxil a small excess is sufficient. In [illegible] of cefatrizine, having an acylatable amino group in the heterocyclic ring, [illegible] of the compound of formula V will be necessary.

[illegible]:
[illegible] most important advantages of the [illegible] previous [illegible] silylation of [illegible] carried out [illegible] the equipment this will favorably influence the output in kilos per batch; the use of a

p-hydroxyphenyl-glycine [illegible] which can only be prepared by a rather difficultly manageable process and which moreover is extremely expensive in a number of countries due to very stringent safety regulations, or indeed permission for its manufacture cannot be obtained at all; the use of large amounts of other additional chemicals is avoided; the desired final product can surprisingly be prepared in economically attractive initial yields and a quality acceptable under available health regulations while at the same time, the number of [illegible] that recovery of [illegible] is rather simple and economically attractive, [illegible] can be easily [illegible]; and [illegible] unexpected crystallization of 6-APA, 7-ACA [illegible] practically [illegible] that a reliable and rather trouble-free process is provided.

BSPR:
The application of the solvent systems during the preparation of the mixed anhydride surprisingly appeared to lead to (a) an improved and [illegible] reproducible and reliable formation of the mixed anhydride and hence an improved conversion yield of the desired compound; (b) an improved yield of the desired compound in combination with a simultaneous increase of the concentrations to an attractive level, with reference to the present economical requirements; (c) the rather unexpected application of N,O-silylated 6-APA and 7-ACA or derivatives thereof for the reaction with the mixed anhydrides in the indicated yields; (d) to improved conversion yields in the case of the application of cosolvents of the amide type for certain conversions of silylated 7-ACA derivatives; and (e) the possibility to carry out the silylation reaction and the preparation of the mixed anhydride in one and the same water immiscible main solvent, and more particularly dichloromethane, as a result of which the presently required recovery of solvents is drastically simplified.

BSPR:
As in industrial processes the application of thoroughly dried solvents and/or Dane salts of very high purity is an ideal that will never be realized completely, a slight excess of the starting Dane salts and of lower alkyl chloroformates are preferably used. In one of the preferred embodiments of the process of the present invention, amoxicillin trihydrate is prepared, starting from dry dichloromethane, 6-APA, [illegible], trimethylchlorosilane in the presence of triethylamine in exactly balanced mutual amounts and in an amount of about two equivalents with reference to 6-APA, of potassium D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate with N-methylmorpholine as catalyst; and a cosolvent selected from N,N-dimethylacetamide, N-methylpyrrolidone, N,N-dimethylformamide, and tetramethylurea or mixtures thereof, and methyl chloroformate, while the solutions of the in situ prepared silylated 6-APA acid and the mixed anhydride are [illegible] temperature of [illegible], followed by recovering amoxicillin by known methods.

DETX:
6-APA [illegible] with an excess of a strong tertiary amine base such as triethylamine in an inert, water-immiscible [illegible] such as methylene chloride [illegible] resulting in a solution of a salt of 6-APA with the base in said solvent,

DETX:
[illegible] hexamethyldisilazane (HMDS) [illegible] 6-APA and after heating [illegible], the mixture was [illegible] hours. The [illegible], which was [illegible], was [illegible]. The [illegible] in the reaction flask was [illegible] by [illegible] small amounts of dichloromethane when necessary. The 6-APA was completely dissolved and after the titration of the starting [illegible] N-silylation [illegible] in a washing [illegible] connected to the reaction vessel,

[illegible] of the starting HAMA [illegible] appeared [illegible] result [illegible].

DETD:
[illegible] 6-APA [illegible] were suspended in [illegible] and [illegible] ml of methylene chloride were added thereto. With stirring, [illegible] ml of triethylamine were added at [illegible] temperature and then [illegible] ml of trimethylchlorosilane (TMCS) were added [illegible] minutes while keeping the temperature [illegible]. After stirring for [illegible] at that temperature, the pH was [illegible] by addition of 4 ml of TMCS, the pH [illegible] with a Radiometer pH meter type TTT1c [illegible] Radiometer GK 2401 C electrode was brought to pH [illegible].

DETD:
In a nitrogen atmosphere, [illegible] g ([illegible] mmoles) of 6-APA were suspended in [illegible] ml of dry methylene chloride and [illegible] ml ([illegible] mmoles) of triethylamine and [illegible] ml ([illegible] mmoles) of trimethylchlorosilane were added subsequently. This mixture was refluxed with stirring for 1 hour and then was cooled in an ice bath to [illegible].

DETD:
With vigorous stirring the turbid solution of the Dane anhydride prepared in Step A and cooled to [illegible].degree. C. was added all at once to [illegible] cooled solution of the silylated 6-APA obtained in Step B and the mixture was stirred for a further [illegible] minutes in a ice bath. Then, the ice bath was removed but stirring was continued with introduction of nitrogen until room temperature was reached (about 40 minutes). The mixture was poured into with cooling 75 ml of ice water, whereafter the pH, which reached a value of 2.5 to 3 was adjusted to 1 to 1.2 with concentrated hydrochloric acid as measured with an Electrofact KCL electrode or 1.0 to 1.2 with an AgCl electrode.

DETD:
In substantially the same manner as described in Example [illegible], amoxicillin trihydrate was obtained in a yield of [illegible] with a mercurimetrically measured purity of [illegible], a biologically measured quality of [illegible] and an optical rotation [.alpha.].sub.D.sup.20 of +[illegible].degree., starting from 42.[illegible] mmoles of potassium D-.alpha.-(1-carbethoxypropen-2-yl)-amino-p-hydroxyphenylacetate in [illegible] ml of methylisobutylketone and [illegible] ml of tetrahydrofuran, [illegible] ml of N-methylmorpholine, 44 mmoles of [illegible] formate in 18 ml of methylisobutylketone, [illegible] mmoles of 6-APA in [illegible] ml of dry methylene chloride, [illegible] mmoles of triethylamine and [illegible] mmoles of trimethylchlorosilane.

DETD:
In substantially the same manner as described in Example [illegible], amoxicillin trihydrate was obtained in a yield of [illegible] having a purity of [illegible] according to hydroxylamine measurement, a biologically measured quality of 94.4 and an optical rotation [.alpha.].sub.D.sup.20 of +[illegible].degree., starting from 47.[illegible] mmoles of potassium D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate in [illegible] ml of methylisobutylketone and [illegible] ml of tetrahydrofuran, [illegible] ml of N-methylmorpholine, [illegible] mmoles of methyl chloroformate (purity [illegible]) in [illegible] ml of methylisobutylketone, [illegible] mmoles of 6-APA in [illegible] ml of dry methylene chloride, [illegible] mmoles of triethylamine and [illegible] mmoles of trimethylchlorosilane. The pH value measured with a Radiometer pH meter TTT 1,C and a Radiometer GK 2401 C electrode was kept constant at [illegible].4 at the end of the silylation reaction.

DETD:
In substantially the same manner as described in Example [illegible], amoxicillin trihydrate was obtained in a yield of [illegible] having a purity of [illegible] according to hydroxylamine measurement, a biologically measured quality of [illegible] and an optical rotation [.alpha.].sub.D.sup.20 of [illegible].degree., starting from [illegible] mmoles of potassium D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate in [illegible] ml of methylisobutylketone and [illegible] ml of N-methylpyrrolidone, [illegible] ml of N-methylmorpholine, 4[illegible] mmoles of methyl chloroformate (purity [illegible]) in [illegible] ml of methylisobutylketone, 4[illegible] mmoles of

6-APA in [illegible] ml of dry methylene chloride, [illegible] mmoles of triethylamine and [illegible] mmoles of trimethylchlorosilane. The pH value measured with a Radiometer pH meter type TTT[illegible] and a Radiometer GK 2401C electrode was adjusted at [illegible] at the end of the silylation reaction.

DEPR:
In substantially the same manner as described in Example [illegible], amoxicillin trihydrate was obtained in a yield of [illegible] having a [illegible] measured purity [illegible], starting from [illegible] mmoles of [illegible] D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate in [illegible] ml of methylisobutylketone and [illegible] ml of tetrahydrofuran, [illegible] ml of N-methylmorpholine, 44 mmoles of ethyl chloroformate in [illegible] ml of methyl isobutylketone, 4[illegible] mmoles of 6-APA in [illegible] ml of dry methylene chloride, [illegible] mmoles of triethylamine and 62 mmoles of trimethylchlorosilane.

DEPR:
[illegible] g of 6-aminopenicillanic acid were weighed into a [illegible] l reaction vessel and 4[illegible] ml of methylene chloride were added thereto. After addition of [illegible] g of bis(trimethylsilyl)-urea, the mixture was refluxed for about 2.5 hours and the mixture was then cooled to 20.degree. C. The "pH" reading, on the scale of a Radiometer pH meter type TTT 2C, connected with a Radiometer GK-2401C electrode, was 6.3.

DEPR:
In the same manner as described in Example 13, 44.3 g of amoxicillin trihydrate having a purity of 97.7% were obtained by reaction of methoxycarbonyl D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate and 35 g of 6-aminopenicillanic acid, previously silylated with 72.5 g of bis(trimethylsilyl) acetamide instead of the bis(trimethylsilyl)urea.

DEPR:
(b) Silylation of 6-APA

DEPR:
[illegible] g of 6-APA are stirred in 4[illegible] ml of methylene chloride. After addition of 45 ml of triethylamine at ambient temperature and under stirring, [illegible] ml of trimethylchlorosilane are added in about [illegible] minutes at a temperature of [illegible].degree.-[illegible].degree. C. After additional stirring for 1 hour the "pH" value is adjusted to a final value of 6.7 by the addition of 4.9 ml of trimethylchlorosilan. The mixture is cooled to -40.degree. C.

DEPR:
In the same manner as described in Examples 13-15, 55.3 g of amoxicillin trihydrate are obtained in a yield of [illegible] having a purity of 94.8% according to a hydroxylamine method measurement, a biologically measured purity of [illegible], a mercurimetrically measured purity of [illegible], and an optical rotation [.alpha.].sub.D.sup.20 of +[illegible], starting from [illegible] g of potassium D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate in 2[illegible] ml of methylene chloride and [illegible] ml of N-methylpyrrolidone, [illegible] ml of N-methylmorpholine, [illegible] ml of methyl chloroformate, 35 g of 6-APA in [illegible] ml of methylene chloride, 45 ml of triethylamine and 35 ml of trimethylchlorosilane. The "pH" value measured with a Radiometer pH meter TTT[illegible], and a Radiometer GK 2401C electrode was adjusted at 6.7 at the end of the silylation reaction, while the solution of the mixed anhydride as well as the solution of silylated 6-APA were both previously cooled to -4[illegible].degree. C. and reacted at -[illegible].degree. C. [illegible]

DEPR:
In the same manner as described in Examples 13-1[illegible], [illegible] g of amoxicillin trihydrate are obtained in a yield of [illegible] having a purity of [illegible] according to a hydroxylamine method measurement, a biologically measured purity of [illegible], a mercurimetrically measured purity of [illegible] and an optical rotation [.alpha.].sub.D.sup.20 of +[illegible], starting from [illegible] g of potassium

D-.alpha.-[illegible] [illegible] N,N-[illegible], [illegible] N-methylmorpholine, [illegible] methyl chloroformate, [illegible] g of 6-APA in [illegible] [illegible] and [illegible] trimethylchlorosilane. The "pH" value measured with a Radiometer pH meter TTT2, and a Radiometer GK [illegible] was adjusted at [illegible] at the end of the silylation reaction, while the [illegible] of the mixed anhydride and of silylated 6-APA were pre-cooled [illegible] and reacted at [illegible] [illegible] [illegible] acylation yield [illegible].

DRIB:
In the same manner as described in examples 13-15, amoxicillin was prepared in a [illegible] acylation yield starting from [illegible] g of potassium D-.alpha.-(1-carbomethoxypropen-2-yl)-amino-p-hydroxyphenylacetate in 400 ml of methylene chloride, [illegible] ml of N,N-dimethylformamide 1.5 ml of N-methylmorpholine, [illegible] ml of methyl chloroformate, [illegible] g of 6-APA in [illegible] ml of methylene chloride, [illegible] ml of triethylamine and [illegible] ml of trimethylchlorosilane. The "pH" value measured with the same equipment as in the preceding examples was adjusted at [illegible] at the end of the silylation reaction, while solutions of the mixed anhydride and of silylated 6-APA were pre-cooled to -40.degree. C. and reacted at -[illegible].degree. C. for 2 hours.

DEPU:
STEP B: Silylation of 6-APA

DEPU:
STEP B: Silylation of 6-APA

DEPU:
STEP B: Silylation of 6-APA

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[illegible] Entry [illegible] File: [illegible] [illegible]

DOCUMENT-IDENTIFIER: [illegible] A
TITLE: [illegible] phenyl [illegible] cephalosporin derivatives

BSPR:
Acylation of the 7-amino group of a cephalosporin, for example, of 7-aminocephalosporanic acid (7-ACA), or of the 6-amino group of a penicillin (6-APA), is a known reaction and any functional equivalent of a compound of Formula (III) which is usually employed as an acylating agent for primary amino groups can be used. Examples of appropriate acylating derivatives [illegible] of the free acid are known. The free acid can be coupled with a compound of Formula (II) [illegible] with one of its readily-cleavable esters, for example, in the presence of an enzyme or of an N,N'-carbonyldiimidazole or of an N,N'-carbonylditriazole or of a carbodiimide reagent, for example, N,N'-diisopropyl carbodiimide, N,N'-dicyclohexyl carbodiimide or N-cyclohexyl-N'-(2-morpholinoethyl)-carbodiimide.

BSPR:
Other usable activated derivatives include the corresponding azolides, i.e., amides of the corresponding acids, the amide nitrogen of which is a member of a quasiaromatic 5-membered ring which contains at least two nitrogen atoms, for example, of an imidazole, pyrazole, triazole, benzimidazole or benzotriazole ring. Another usable derivative of a substituted phenylglycine of Formula (III) is a N-carboxyanhydride (Leuchs' anhydride). The group which activates the carboxyl group here also protects the amino group.

BSPR:
For example, 7-ACA tert.-butyl ester or 6-APA tert.-butyl ester can be reacted with a compound of Formula (V) ##STR1## wherein R[illegible] is a protecting group which can be split off and R, A and [illegible] are as above, with a hydroxybenzotriazole [illegible] and with DCC in substantially equimolar proportions, with [illegible], in an inert solvent, especially in a chlorinated hydrocarbon, such as methylene chloride; in an ether, such as THF; or in an aprotic dipolar solvent, such as DMF or DMSO; or in a solvent mixture.

BSPR:
Reaction of a compound which otherwise corresponds to Formula (I), but in which R is hydrogen and A is a [illegible] group, with an alkylating agent [illegible] in the presence of an acidic catalyst, preferably [illegible] acid, such as hydrochloric, hydrobromic [illegible], or of an organic acid, especially of a sulfonic acid, such as p-toluenesulfonic acid.

DETX:
The following abbreviations are used in the Examples: DMF dimethylformamide, 7-ACA 7-aminocephalosporanic acid, 6-APA 6-aminopenicillanic acid, DCC dicyclohexyl carbodiimide, THF tetrahydrofuran, BOC tert.-butyloxycarbonyl, [illegible]oxycarbonyl-1-methylvinyl, [illegible]-phenyl-[illegible]-4-carboxylic acid, [illegible]-4-carboxylic acid, 7A [illegible] acid, 6A-penicillanic acid.

DETX:
[illegible] substituted phenylglycine of Formula (III), [illegible] are used in the form of racemates. The corresponding compounds of Formula (I) are then obtained as mixtures of two

[illegible].

[illegible]:
6-APA triethylamine salt [illegible] g. dissolved in [illegible] ml. of dichloromethane is
added dropwise at -[illegible].degree. C. to a solution of
ethyl-2-[illegible]-(1-ethoxycarbonyl-1-methylvinylamino)-2-p-ethoxycarbonyloxypheny
[illegible] carbonate, prepared in situ from [illegible] g. of
[illegible]-1-methylvinylamino)-2-p-hydroxyphenylacetic acid and
[illegible]
[illegible]
[illegible]
[illegible] extracted with [illegible] ml. of water. The [illegible]
[illegible] with [illegible] aqueous hydrochloric acid, extracted with ethyl
[illegible] and allowed to stand overnight. Precipitation with petroleum ether
[illegible]-amino-p-ethoxycarbonyloxyphenylacetamido)-PA; R.sub.f [illegible]
silica gel [illegible]:water=[illegible]:15 v/v).

WEST

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[illegible] [illegible] [illegible]

[illegible]-IDENTIFIER: [illegible] A
TITLE: Acylation [illegible] penicillins and [illegible]

ABPL:
A [illegible] .beta.-lactam [illegible] by enzyme catalyzed acylation [illegible] the parent .beta.-lactam with an activated derivative of the side chain acid wherein a modulator, which consists of one or more compounds different from the reactants and the reaction product and which suppresses the hydrolysis of the activated derivative of the side chain acid and the desired product more than it suppresses the synthesis of the desired product, is added to the reaction mixture, at the beginning of the reaction process, in a concentration from about [illegible] to [illegible].times.10.sup.3 .mu.m.

BSPR:
The present invention relates to an improved method for enzymatic acylation. In particular, the invention relates to the preparation of .beta.-lactam antibiotics by enzymatic acylation of the parent amino .beta.-lactam moiety with an acylating agent which is an activated derivative of the side chain acid.

BSPR:
Enzymatic production of semisynthetic .beta.-lactam antibiotics by acylation of the parent amino .beta.-lactam moiety with the side chain acid or an activated derivative, such as an amide or an ester thereof, is known e.g. from West German patent application having publication No. 2,[illegible], Austrian Patent No. [illegible], Dutch patent application No. [illegible], West German patent application having publication No. [illegible], European patent application having publication No. [illegible], international patent application having publication No. WO [illegible] and from international patent application having publication No. WO [illegible].

BSPR:
The parent amino .beta.-lactams such as 6-aminopenicillanic acid (6-APA) and 7-amino[illegible]cephalosporanic acid (7-ADCA) are commonly produced by [illegible] hydrolysis of a fermented penicillin, for example penicillin V or [illegible]. [illegible] impurities originating from the fermentation, the [illegible] typically contains [illegible] traces of the [illegible] .beta.-lactam [illegible] used as starting material at a concentration of [illegible] can be purified and crystallized to obtain pure 6-APA or 7-ADCA [illegible] the 7-ADCA case, the fermented penicillin has to go through a [illegible] process before the hydrolysis step).

BSPR:
[illegible] of the known methods for enzymatic production of .beta.-lactam [illegible] with an activated [illegible] conditions used [illegible] part of the acylating agent hydrolyses before it has reacted with the amino .beta.-lactam. Thus, when the amide of the side chain acid is used as acylating agent, some free side chain acid and an equivalent amount of ammonia will be generated in the reaction mixture as a result of this hydrolysis. Similarly, when an ester of the side chain acid is used as acylating agent, some free side chain acid and an equivalent amount of the alcohol corresponding to the ester will be generated in the reaction mixture as a

result of the hydrolysis. Also, the desired product formed hydrolyses to [illegible] free side chain acid and the parent amino .beta.-lactam.

BSPR:
It has now, surprisingly, been found that certain modulators, i.e. compounds different from the reactants and the reaction product, can be added to a reaction mixture in which enzymatic synthesis of a .beta.-lactam antibiotic takes place in a concentration which is lower than that of the reactants, preferably lower than about 100 mM, to suppress the reaction in such a way that the hydrolysis of the acylating agent--when an activated derivative of the side chain acid is used as acylating agent--and of the desired product is suppressed more than the synthesis of the desired product.

BSPR:
Accordingly, in its broadest aspect the present invention relates to a method of producing a semisynthetic .beta.-lactam antibiotic by enzymatic acylation of the parent amino .beta.-lactam with an activated derivative of the side chain acid wherein a modulator which suppresses the hydrolysis of the acylating agent and the desired product more than it suppresses the synthesis of the desired product is added to or present in the reaction mixture.

BSPR:
Examples of .beta.-lactam antibiotics which can be produced by the process of this invention are ampicillin, amoxicillin, ticarcillin, cefaclor, cefatrizine, cefaparol, cephradine, cephalexin, cefadroxil, cephaloglycin and cephalothin.

BSPR:
The acylating agent to be used in the method of this invention is an activated derivative of the side chain acid such as a lower alkyl (methyl, ethyl, n-propyl or isopropyl) ester or an amide. The amide can be unsubstituted in the --NH.sub.2 group which is preferred, or it can be substituted by one or two lower alkyl groups--identical or different--selected from the group comprising methyl, ethyl, propyl and isopropyl. The derivative may be used in the form of a salt, for example, the hydrochloride or the sulphate. Examples of side chain acids are D-phenylglycine or D-p-hydroxyphenylglycine.

BSPR:
Examples of parent amino .beta.-lactams which can be acylated by the method of this invention are 6-aminopenicillanic acid (6-APA), 7-aminocephalosporanic acid (7-ACA), 7-aminodesacetoxycephalosporanic acid (7-ADCA) and 7-amino-3-chloro-3-cephem-4-carboxylate.

DEPR:
The amount of modulator to be added to the reaction mixture in order to obtain the desired effect depends i.a. on the identity of the modulator and on the amount of enzyme present in the [illegible]. However, some guidance to this can be found in the Examples and in the claims. It is thus important to notice that a too high concentration of the modulator will prevent the desired reaction from taking place. Under all circumstances the useful concentration of modulator in the reaction mixture is so low that it does not influence the water activity in the reaction mixture. In any case, it will be lower than that of the reactants, preferably lower than 100 mM.

DEPR:
The enzyme to be used in the process of this invention may be any enzyme catalyzing the reaction in question. Such enzymes have been known since around [illegible]. [illegible] enzymes [illegible], for example, termed penicillin amidase or [illegible] penicillin acylases and classified as E.C. [illegible]. A number of microbial enzymes are known to have this activity, derived from for example Acetobacter, Xanthomonas, Mycoplana, Protaminobacter, Aeromonas (West German patent application having publication No. [illegible]), Pseudomonas (Austrian Patent No. [illegible]), Flavobacterium (Dutch patent application No. [illegible]), Aphanocladium, Cephalosporium (West German patent application having publication No. [illegible]), Acetobacter pasteurianum, Bacillus megaterium,

[illegible] patent application having publication No. [illegible], Kluyvera citrophila [illegible] and Escherichia coli West German patent application having publication No. [illegible]. The Escherichia coli enzyme is commercially available. The enzyme may also be a so-called ampicillin hydrolase, acylase or amidase. In this connection, reference is, inter alia, made to Hakko to Kogyo 38 [illegible], 216 et seq., the contents of which is incorporated by reference.

[illegible]:
[illegible] enzyme [illegible] form, for example, in [illegible] form. Immobilization may be done by any known method. Immobilized Escherichia coli enzyme is commercially available from Boehringer Mannheim GmbH, Germany, under the trade name Enzygel.

[illegible]:
The solubility of the acylating agent such as the D-phenylglycine or D-p-hydroxyphenylglycine derivative [illegible] the D,L mixtures as described in [illegible], will vary with the [illegible] of the derivative and with the [illegible] of the reaction medium. In an aqueous system as used in the examples, the solubility of the hydrochloride of D-phenylglycine amide is typically approximately 450 mM. However, the solubility is very dependent on the salt components in the solution, as well as on the pH value and the temperature of the solution. In some embodiments of the process of this invention, the initial reaction mixture is a slurry containing undissolved acylating agent and/or amino .beta.-lactam, which will dissolve partly or fully during the course of the reaction. The .beta.-lactam antibiotic formed may precipitate during the reaction and, also, the hydrolysis products of the acylating agent such as D-phenylglycine and D-p-hydroxyphenylglycine, may precipitate. Hence, in some cases the reaction mixture will be a slurry throughout the duration of the reaction.

DEPR:
The amino .beta.-lactam, for example 6-APA or 7-ADCA, used in the process of this invention may be obtained by enzymatic hydrolysis of the fermented penicillins or cephalosporins (for example penicillin V, penicillin G or [illegible] ring-enlarged analogues (for example V-DCA and [illegible]) [illegible] the hydrolysis [illegible] phenoxyacetic acid etc.). In some cases, the [illegible] solution can be used directly without further purification or dilution.

TEPR:
Generally, the reaction temperature of the process of this invention may vary between about 0.degree. C. and about 35.degree. C., especially between about 5.degree. C. and about 30.degree. C. Temperatures in the range about [illegible].degree.-30.degree. C. may be preferred for convenient operation. The pH value which is optimal depends on the type and purity of enzyme. Using [illegible] enzyme, the optimal pH value is typically in the range from about [illegible] to about [illegible], preferably in the range from about [illegible] to about [illegible]. For the preparation of amoxicillin, a pH value in the range from about 5.5 to about 6.4 is preferred. Control of the pH value may be used. Suitable reaction times are from several minutes to several hours, in particular from about [illegible] hour to about [illegible] hours. Suitable enzyme concentrations may be from about [illegible] ml to about [illegible] ml [illegible] unit of enzyme activity, see below).

[illegible]:
[illegible] this invention, unusually high yields of the [illegible] .beta.-lactam [illegible] obtained. The high yields are [illegible] [illegible] this invention [illegible] selecting the concentration of the acylating agent, the ratio between the concentration of acylating agent and the starting amino .beta.-lactam, the pH value, the enzyme and the [illegible] and amount of [illegible].

[illegible]:
D-HPGA is D-p-hydroxyphenylglycine amide, D-HPG is D-p-hydroxyphenylglycine, 6-APA is 6-amino penicillanic acid, Amox is amoxicillin, Phox is phenoxyacetic

acid, and Phyl is phenylacetic acid.

[illegible]:
[illegible] Amox [illegible] formation, and [illegible] D-HPG [illegible] D-HPG formation, [illegible] enzyme, [illegible] enzyme activity).

[illegible]:
In the following [illegible] of penicillin G acylase activity is used: one unit (U) corresponds to the amount of enzyme that hydrolyses per minute 1 [illegible] of penicillin G under standard conditions (5% penicillin G, 0.2M sodium phosphate buffer, pH 8.0, 28.degree. C.).

[illegible]:
The ratio X is defined as the number of moles of D-HPGA consumed per mole of Amox produced. For practical use this can be transformed to X=(moles D-HPG+moles Amox)/moles Amox, wherein "moles D-HPG" is the molar amount of D-HPG produced by hydrolysis of the acylating agent and the desired product and "moles Amox" is the molar amount of Amox present in the reaction mixture. Thus, if X is 1 this means that only the desired synthesis takes place, no hydrolysis. If X is 2, this means that D-HPG and Amox are formed in equal amounts (on a molar basis). If X is 3, this means that twice as much D-HPG as Amox is present in the reaction mixture (on a molar basis). The ratio X can be calculated at any time during reaction, but in the following examples X is calculated at the reaction stop time, which is defined as the time at which 90% of the theoretical yield of Amox is present in the reaction mixture (based on the inserted amount of 6-APA). Square brackets are used to designate molar concentrations.

DEPR:
Retention times in minutes: 2.6 (D-HPG), 3.5 (D-HPGA), 5.0 (6-APA), 13.5 (Amox).

DEPR:
In Examples 1-5 the following standard conditions for enzymatic amoxicillin synthesis have been used (see patent application No. WO 92/01061 for further details):

[illegible]:
During the reactions, the pH value of the reaction mixtures was kept constant by titration with [illegible]N sulphuric acid.

DEPR:
A standard synthesis (immobilized penicillin G acylase from E. coli; enzyme dosing 5.6 U/ml) was carried out with no Phox added. The Phox level was [illegible] .mu.M in the reaction mixture due to a residual Phox content of [illegible]% w/w in [illegible]. The results are reported in Table 1.

[illegible]:
A standard synthesis (immobilized penicillin G acylase from E. coli; enzyme dosing 5.6 U/ml) was carried out with no Phyl added. The Phox level in the reaction mixture was [illegible] .mu.M. The results are reported in Table 2.

[illegible]:
Four different immobilized Pen G acylase preparations were used: a) immobilized penicillin G acylase from E. coli; enzyme dosing 5.6 U/ml; b) [illegible], dosing [illegible] U/ml; c) immobilizate obtained from [illegible], dosing [illegible] U/ml; and d) immobilizate obtained from Boehringer Mannheim (experimental preparation), dosing [illegible] U/ml.

[illegible]:
[illegible] Pen G acylase preparations were obtained from [illegible], art. No. [illegible], [illegible] U/ml, and from [illegible], [illegible] U/ml. An enzyme dosage of [illegible] U/ml were employed in the two series. The results are reported in Table [illegible].

DETD:
A standard synthesis [illegible] immobilized penicillin G acylase from E. coli; enzyme [illegible] was carried out with [illegible]-mandelic acid added. The Phox [illegible] level was [illegible] mM in the reaction mixture. The results obtained [illegible] in Table [illegible].

DETD:
A standard synthesis [illegible] Boehringer Mannheim immobilized penicillin G acylase, [illegible] enzyme [illegible] was carried out with [illegible]. The Phox [illegible] level in the reaction mixture was [illegible] mM. The results obtained are [illegible] in Table [illegible].

DETD:
[illegible] improve synthesis [illegible] standard synthesis [illegible], immobilized penicillin G acylase from E. coli; enzyme [illegible] when they are present in the reaction mixture in a [illegible] within the interval [illegible], synthesis of the desired [illegible]-lactam antibiotic is favoured as compared to hydrolysis of the acylating agent:

DEPC:
Enzyme Activity

DEPC:
Enzymatic Synthesis of Amoxicillin Using a Fixed Dosage of Immobilized Pen G Acylase and Varying the Phox Concentration in the Reaction Mixture from 2.6 to [illegible] mM

DEPC:
Enzymatic Synthesis of Amoxicillin Using a Fixed Dosage of Immobilized Pen G Acylase and Varying the Phyl Concentration in the Reaction Mixture from [illegible] to [illegible] mM

DEPC:
Enzymatic Synthesis of Amoxicillin Using Various Immobilized Preparations of Pen G Acylase and Varying the Phox Concentration in the Reaction Mixture from [illegible] to [illegible] mM

DEPC:
Enzymatic Synthesis of Amoxicillin Using Varying Amounts of Enzyme and a Constant Concentration of Phox

DEPC:
Enzymatic Synthesis of Amoxicillin by Using Soluble Pen G Acylase (Two Different Suppliers) and Varying the Phox Concentration in the Reaction Mixture from [illegible] to 14[illegible] mM

DEPC:
Enzymatic Synthesis of Amoxicillin by Using Immobilized Pen G Acylase and Varying the Concentration of [illegible]-Mandelic Acid from [illegible] to [illegible] mM

DEPC:
Enzymatic Synthesis of Amoxicillin by Using Immobilized Pen G Acylase and Varying the [illegible]-Thiophene[illegible] Acid Concentration from [illegible] to [illegible].3 mM in the Reaction Mixture

DEPC:
Enzymatic Synthesis of Amoxicillin Using Immobilized Pen G Acylase and Var[illegible] [illegible]

DETL:
TABLE 4 Initial velocity (Phox) [illegible] mM Enzyme [illegible] Experiment mixture [illegible] ml Amox [illegible]-HPG time A 3[illegible] 11.[illegible] 1.1[illegible] [illegible] 1.[illegible] B 3[illegible] 2[illegible] [illegible]

[illegible]

[illegible]: [illegible] Initial velocity Enzyme [illegible]
[illegible]
[illegible] reaction [illegible]
reaction [illegible] mixture Amox [illegible] stop time
[illegible]
[illegible]
[illegible]

CLMS:
1. A method for providing a semisynthetic .beta.-lactam antibiotic by enzyme catalyzed acylation of the parent .beta.-lactam with an amide or ester of the side chain acid wherein a modulator, which is a carboxylic acid of 2 to 2[illegible] carbon atoms, and is different from the reactants and the reaction product is added to the reaction mixture, at the beginning of the reaction process, in a [illegible]

[illegible]:
Segel. Enzyme Kinetics, [illegible].

OREF:
McDougall et al. Enzyme Microb. Technol. 1982 vol. 4, pp. 114-115.

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[illegible] Entry [illegible] of [illegible] File: [illegible] Apr [illegible]

[illegible]-IDENTIFIER: [illegible] A
TITLE: [illegible] methyl [illegible] of penicillins

[illegible]:
[illegible] preferably salts with pharmaceutically acceptable inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, tartaric acid, citric acid, fumaric acid, and the like.

[illegible]:
In contrast to many of the corresponding free penicillins, the compounds of the invention are [illegible] absorbed from the gastrointestinal tract and are then, under the influence of enzymes, rapidly hydrolysed to the corresponding free penicillins. This hydrolysis is an important feature of the compounds of the invention. It is assumed that the first step consists in a hydrolysis to the hydroxymethyl esters of the corresponding penicillins which subsequently decompose spontaneously to the free penicillins.

BSPR:
The compounds of the above formula VIIIa are new and can be prepared in different manners, for instance by reacting 6-aminopenicillanic acid with a compound of the formula ##EQU[illegible]## in which R.sub.[illegible], R.sub.[illegible], [illegible], and n are as [illegible] above. The reaction is preferably performed in the presence of an amine, e.g. triethylamine, and at room temperature or slightly elevated temperatures in an inert solvent, such as dimethylformamide, whereby an intermediate of the formula ##EQU[illegible]## can be isolated which, after conversion of the group [illegible] into an unsubstituted or substituted amino group, yields the compounds of formula VIIIa. The conversion of [illegible] can be performed as described hereinbefore. The amino group at the 6-position of the penicillanic acid can be, but is not necessarily, protected by well-known protecting groups, for instance [illegible].

[illegible]:
[illegible] Amino Acid R.sub.[illegible] [illegible] M.p. [illegible] [illegible] 1. L-valine [illegible]CH.sub.[illegible].sub.[illegible] [illegible] 2. [illegible]-valine -- [illegible] -- 3. D,L-valine -- [illegible] -- 4. L-leucine [illegible] [illegible] 5. L-isoleucine C.sub.2 [illegible]H.sub.[illegible].sub.2.CH.CH.sub.2 -- [illegible] 114-11[illegible] 6. D,L-tert.leucine [illegible]CH.sub.[illegible].sub.3 C-- [illegible] H.sub.[illegible].CH.CH.sub.[illegible] [illegible] 7. L-aspartic acid --[illegible]COOCH.sub.[illegible] [illegible] 154-15[illegible] .alpha.-methyl ester* [illegible] 8. L-aspartic acid --CH.sub.[illegible]COOCH.sub.3 [illegible] 112-114 .beta.-methyl ester** [illegible]. [illegible] 10. D-.alpha.-phenylglycine C.sub.6 H.sub.5 -- [illegible] [illegible]
[illegible] *[illegible].Am.Chem.Soc. [illegible], [illegible]
**[illegible].Am.Chem.Soc. [illegible], [illegible]

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[illegible]

[illegible]

[illegible]

ABPL:
Process for the recovery of ampicillin from a mixture containing ampicillin and 6-aminopenicillin acid (6-APA), in which a mixture of ampicillin and 6-APA, with a pH higher than 7, which apart from any solid ampicillin being present is homogeneous at a pH between 7 and 8.5, is subjected to a pH lowering till a pH lower than 6.2 is reached, and the solid substance present is recovered. The process is in particular suitable to be applied to the [illegible] mixture which is obtained after the enzymatic acylation reaction of 6-APA with a [illegible] derivative as acylation agent. Pure ampicillin can thus be recovered in a simple way.

BSFR:
The invention relates to a process for the recovery of ampicillin from a mixture containing ampicillin and 6-aminopenicillic acid (6-APA).

BSFR:
In the preparation of ampicillin, with 6-APA being acylated with a [illegible] derivative, the recovery of the ampicillin and working up of the [illegible] mixture are difficult in general.

BSFR:
A process for isolating the ampicillin pure from a mixture containing ampicillin and minor quantities of 6-APA is described in JP-A-47030687. According to the process described in this Japanese publication, an acid aqueous mixture containing 6-APA and ampicillin is subjected to an extraction with butanol or isoamylalcohol, after which the pH is raised to a value between [illegible] and [illegible] and the product is recovered by complete boiling down and freeze-drying. [illegible] drawback of this method is that organic solvents that are [illegible] to the process have to be added. In addition, complete boiling down and freeze-drying is not industrially practicable. Moreover, the process involves formation of salts that are included in the freeze-dried product.

BSFR:
JP-A-[illegible] discloses a process wherein ampicillin is recovered from a mixture of ampicillin and amino-penicillanic acid by conversion of the ampicillin to the trialkylamine salt and recover the ampicillin as its trialkylamine salt.

BSFR:
[illegible] simple, industrially practicable [illegible] ampicillin [illegible] making use of [illegible] organic solvents that are alien to the process.

BSFR:
This is achieved according to the invention in that a mixture containing ampicillin and 6-APA and having a pH higher than 7, which, apart from any solid ampicillin that is present, is homogeneous at a pH between 7 and [illegible], is subjected to a pH lowering to a pH lower than [illegible], and that the solid substance present is recovered.

BSFR:

[illegible] pH [illegible] lower than [illegible] between [illegible] and [illegible], [illegible] mixture, [illegible] [illegible] than [illegible] mass [illegible], in ampicillin [illegible] with a purity [illegible] amount of 6-APA is present in particular more than [illegible] mass [illegible], even if a large [illegible] the mixture, after which it can be recovered. Besides ampicillin the reaction mixture often contains other valuable components, such as for instance the 6-APA. In order to get a commercially attractive process it is consequently [illegible] also [illegible] the 6-APA and ampicillin losses. It has also [illegible] [illegible] that if subsequently the pH is lowered further to a value of less than [illegible], in particular between [illegible] and [illegible], 6-APA and the remaining ampicillin [illegible] after which this mixture of 6-APA and [illegible], for instance by filtration. The resulting mixture [illegible] 6-APA and ampicillin [illegible] with no significant losses of 6-APA and ampicillin.

DETD:
The process according to the invention is in particular suitable to be applied
in the working up of the reaction mixture which is obtained after the
enzymatic acylation reaction in which 6-APA is acylated with D-phenylglycine
amide [illegible] D-phenylglycine. Thus, the process according to the
invention [illegible] be applied [illegible] a starting mixture that is obtained
by successively filtering, with isolation of immobilized enzyme, the
reaction mixture of an enzymatic acylation reaction carried out at a
relatively high pH, for instance a pH between [illegible] and 10, in particular
between [illegible] and 10, lowering the pH to a value between 7 and 9. Depending on the
amount of D-phenylglycine (PG) formed during the acylation reaction, if
desired, it is possible to remove first at a higher pH-value between 7 and
9--which is selected dependent on the mixture such that already PG has been
crystallized out and ampicillin has not yet--the eventually formed solid
substance, which mostly will consist mainly of PG.

DETD:
[illegible] starting mixture used is the mixture obtained after
an enzymatic acylation reaction that ends at a relatively low pH, for instance
a pH between [illegible] and [illegible], preferably between 7.[illegible] and [illegible], and after isolation of
the solid substance which mainly contains the immobilized enzyme and
D-phenylglycine.

DETD:
[illegible] starting mixture containing 6-APA and ampicillin, a significant [illegible] 6-APA [illegible] 6-APA [illegible] 6-APA [illegible] ampicillin, preferably [illegible], in particular [illegible].

DETD:
The pH may be lowered in several ways in the framework of the invention, [illegible]
instance chemically by adding an acid, for instance a mineral acid, in
particular sulphuric acid, hydrochloric acid or nitric acid. Another
possibility is for instance, if PGA has been used as acylation agent in the
[illegible] has been used and the pH has been [illegible] with ammonia during the acylation reaction, [illegible] physical removal [illegible] steam [illegible] an inert gas; steam [illegible] in particular thin-film evaporation; [illegible] or electrodialysis. [illegible] spray [illegible]; gas [illegible]

DETD:
[illegible] pH at which ampicillin is [illegible] 6-APA and ampicillin [illegible] ampicillin [illegible] ampicillin [illegible] ampicillin [illegible] 6-APA still completely in solution, and [illegible]

[illegible] pH in a given situation.

BSPR:
The process according to the invention for recovery of pure ampicillin in combination with recirculation of the mixture of 6-APA and ampicillin obtained after further pH [illegible], applied to the mixture obtained after enzymatic acylation of 6-APA [illegible] 6-APA [illegible] higher than [illegible]. The remaining filtrate, which mainly contains [illegible] amounts of PGA, may optionally be worked up further, for instance by bringing it to a pH higher than 7, in particular between 7.5 and [illegible]. If desired, it is possible to apply further concentration and cooling to a temperature lower than 10.degree. C., for instance between 0 and 5.degree. C. In this way a process is obtained with which both 6-APA and PGA can be applied with a high efficiency. In the enzymatic acylation reaction, PGA or esters of PGA for instance may be used as acylation agent.

BSPR:
In principle any enzyme can be used that is suitable as catalyst in the coupling reaction. Such enzymes are for instance the enzymes that are known under the general designations 'penicillin amidase' and 'penicillin acylase'. Examples of suitable enzymes are enzymes derived from Acetobacter, Aeromonas, Alcaligenes, Alcaligenes, Aphanocladium, Bacillus sp., Cephalosporium, Escherichia, Flavobacterium, Kluyvera, Mycoplana, Protaminobacter, Pseudomonas and Xanthomonas, in particular Acetobacter pasteurianum, Bacillus megaterium, Escherichia coli and Xanthomonas citrii.

BSPR:
Preferably an immobilized enzyme is used, since the enzyme can be easily isolated and re-used then. Immobilized enzymes are known as such and are commercially available. Example of suitable enzymes are the Escherichia coli enzyme from Boehringer Mannheim GmbH, which is commercially available under the name 'Enzygel.RTM.', the immobilized Penicillin-G acylase from Recordati, the immobilized Penicillin-G acylase from Pharma Biotechnology Hannover, and an Escherichia coli penicilline acylase isolated as described in WO-A-92/12782 and immobilised as described in EP-A-222462.

BSPR:
The enzymatic acylation reaction is mostly carried out at a temperature lower than 40.degree. C., preferably between 0 and 35.degree. C. The pH at which the enzymatic acylation reaction is carried out is mostly between 5.5 and 10, preferably between 6 and 9.

BSPR:
In practice the enzymatic acylation reaction and the working up of the reaction mixture are mostly carried out in water. Optionally, the reaction mixture may also contain an organic solvent or a mixture of organic solvents, preferably less than 30 vol. %. Examples of organic solvents that can be used are alcohols with 1-7 carbon atoms, for instance a monoalcohol, in particular methanol or ethanol; a diol, in particular ethylene glycol or a triol, in particular glycerol.

BSTT:
AMPI ampicillin

BSTT:
AMPI.3H.sub.2 O=ampicillin trihydrate

BSTT:
6-APA 6-aminopenicillanic acid

BSTT:

[illegible]

[illegible]:
[illegible]

[illegible]:
Enzymatic coupling of [illegible] mM of PGA and [illegible] mM of 6-APA at [illegible].degree. C., [illegible]

[illegible]:
A mixture [illegible] 6-APA and [illegible] of PGA was suspended in [illegible] [illegible] The resulting suspension was added to [illegible] [illegible]. This enzyme is [illegible] enzyme [illegible]; [illegible] with [illegible] water.

[illegible]:
After [illegible] the pH had risen to [illegible]. By means of [illegible] NH.sub.3 the pH was brought to [illegible] and after [illegible] minutes the reaction mixture was filtered through a G-3 glass filter; the residue was washed with [illegible] ml of water [illegible].degree. C. . This residue was a mixture of enzyme and PG formed [illegible].

[illegible]:
Enzymatic coupling of [illegible] mM of PGA and [illegible] mM of 6-APA at 5.degree. C., followed by working up.

DEPR:
A mixture of 43.9 g of 6-APA and 30.6 g of PGA was suspended in 877 ml of water and cooled to 5.degree. C. The resulting suspension was added to 100 g of 'wet' immobilized Pen-G acylase from Recordati (Milan). This enzyme is commercially available in a mixture of water and glycerol ('wet enzyme'); before use it was washed three times with 100 ml of water.

[illegible]:
After [illegible] hours the pH had risen to [illegible]. By means of concentrated aqueous NH.sub.3 the pH was brought to [illegible].6 and after [illegible] minutes the reaction mixture was filtered through a G-3 glass filter; the residue was washed with [illegible] ml of water ([illegible].degree. C.). This residue was a mixture of enzyme and PG formed during the reaction.

[illegible]:
1. A process for recovering ampicillin from a mixture containing ampicillin and [illegible] penicillin and 6-APA comprising:

[illegible]:
4. A process according to claim 1, wherein the mixture contains [illegible] mol [illegible] 6-APA, calculated relative to the total amount of 6-APA and ampicillin.

[illegible]:
[illegible]. A process according to claim 1, wherein the mixture contains [illegible] mol [illegible] of 6-APA, calculated relative to the total amount of 6-APA and ampicillin.

[illegible]:
[illegible]. A process according to claim 1, wherein the process further comprises [illegible] the pH [illegible] liquid phase remaining after recovering [illegible] ampicillin [illegible] a second solid substance.

[illegible]:
[illegible]. A process for recovering ampicillin from a mixture containing ampicillin and [illegible] penicillin and 6-APA comprising:

[illegible]:
[illegible]. A process according to claim [illegible], wherein the enzyme [illegible]

CLPR:

11. A process according to claim 10, wherein in the enzymatic acylation [illegible] the pH is maintained [illegible] and wherein said reducing the pH is accomplished by removing ammonia.

CLPV:

providing the mixture containing ampicillin and 6-APA, said mixture having an initial pH greater than 7 and [illegible] at a pH between 7 and [illegible] ampicillin present; and

CLPV:

lowering the initial pH of the mixture to a pH value of [illegible] and [illegible] ampicillin.

CLPV:

obtaining a mixture containing ampicillin and 6-amino penicillin that is prepared from the reaction mixture of an enzymatic acylation reaction in which 6-APA is acylated using D-phenylglycineamide (PGA) or esters of D-phenylglycine, said mixture having an initial pH greater than 7; and

CLPV:

reducing the pH of said mixture and crystallizing out ampicillin.

WEST

Generate Collection

[illegible]

[illegible]
TITLE: Process for preparation of .beta.-lactams utilizing a combined concentration of acylating agent plus .beta.-lactam derivative of at least 400 mM

ABPL:
.beta.-lactam derivatives are synthesized by an enzymatic reaction of the parent amino .beta.-lactam with the corresponding acylating agent, the concentration of the acylating agent plus the concentration of .beta.-lactam derivative in the reaction mixture being above about 400 mM.

BSPR:
This invention relates to a process for the preparation of .beta.-lactam derivatives by enzymatic acylation of the parent amino .beta.-lactam with an acylating agent. The amino .beta.-lactam may be 6-aminopenicillanic acid (6-APA), 7-aminodesacetoxycephalosporanic acid (7-ADCA), 7-aminocephalosporanic acid (7-ACA) or 7-amino-3-chloro-3-cephem-4-carboxylate and the acylating agent may be a derivative of D-phenylglycine or D-p-hydroxyphenylglycine.

BSPR:
Today, semisynthetic .beta.-lactams such as Ampicillin, Amoxicillin, Cefaclor, Cephalexin, Cephadroxil and Cephaloglycin are prepared in industry by chemical methods, for example by reacting an amino .beta.-lactam such as 6-aminopenicillanic acid, usually having its carboxyl group protected, with an activated side chain derivative, followed by the removal of the protecting group by hydrolysis. It is important due to, for example, yield, that the amino .beta.-lactam, for example 6-APA, is used in a pure, dry form, preferably in a purity higher than [illegible]. For example, Ampicillin [illegible] can be prepared by [illegible] 6-APA, having a suitable protected carboxyl group, with D-phenylglycine [illegible], followed by removal of the protecting group by hydrolysis. These reactions typically involve costly steps such as sub zero [illegible] conditions and organic solvents like methylene chloride and [illegible].

BSPR:
Enzymatic [illegible] of Ampicillin from pure 6-APA and a D-phenylglycine [illegible] is known from West German patent [illegible], Austrian Patent No. [illegible], West German patent application having publication No. [illegible] and European patent application having publication No. [illegible] typically used [illegible] mM of the D-phenylglycine derivative and below [illegible] mM of 6-APA, the highest yield reported was [illegible] European patent application having publication No. [illegible].

BSPR:
The amino .beta.-lactam such as 6-APA is normally produced by enzymatic [illegible]

[illegible] mM. [illegible] and crystallized [illegible] 6-APA or 7-ADCA [illegible] 7-ADCA [illegible], the [illegible] penicillin have been [illegible] in the hydrolysis step [illegible].

BSPR:
The potential drawbacks of the known enzymatic methods for production of Ampicillin, Amoxicillin and Cephalexin (none have yet been upscaled to industrial applicability) are the high costs, yield losses and investments [illegible] amino .beta.-lactam is [illegible] used as raw material for the [illegible] .beta.-lactam. Furthermore, the starting [illegible] of the 6-APA are very low (typically less than [illegible] mM), thus [illegible] the formed Ampicillin more difficult and thus more [illegible]. Also, a higher yield in the enzymatic formation of Ampicillin is [illegible].

BSPR:
A process for enzymatic synthesis of Amoxicillin is described in Agric. Biol. Chem. 44 [illegible], [illegible] et seq., which process is performed in a reaction medium containing [illegible] volume/volume or more of [illegible]-propanol and [illegible] volume/volume of other alcohols. When [illegible] of the last mentioned alcohols or [illegible] of [illegible]-propanol are used, the initial concentration of the starting materials, D-.alpha.-(p-hydroxyphenyl)glycine methyl ester and 6-aminopenicillanic acid, is very low, i.e. [illegible] and [illegible] mM, respectively. When [illegible] of 2-propanol is used, the-initial concentration of the starting materials, D-.alpha.-(p-hydroxyphenyl)glycine methyl ester and 6-aminopenicillanic acid, is 460 and 230 mM, respectively. It is stated in this paper that the addition of more than 100 mM of D-.alpha.-(p-hydroxyphenyl)glycine methyl ester and of more than 50 mM of 6-aminopenicillanic acid markedly suppressed the ration of conversion of 6-aminopenicillanic acid into amoxicillin. The conclusion of this statement is that this publication teaches away for increasing the [illegible] .beta.-lactam and of the acylating agent in the reaction mixture.

BSPR:
After the effective filing date of the application for a patent on this invention, namely Sep. 18-21, 1990, a poster was published at a NATO Workshop. The poster dealt with the preparation of cephalosporins and, according to this poster, working at low temperature had several positive effects on the [illegible]. The highest concentration of acylating agent used by this work was [illegible] mM of D-.alpha.-phenylglycine methyl ester and there was no indication [illegible] would be advantageous to use a higher concentration of the acylating agent.

BSPR:
It has now, surprisingly, been found that the yield in the enzymatic preparation of .beta.-lactam derivatives can be improved by carrying out the reaction at high concentrations of the acylating agent.

BSPR:
By the process of this invention, it is possible and attractive to use a crude [illegible] amino .beta.-lactam, for example 6-APA or 7-ADCA, without [illegible] amino .beta.-lactam, for example 6-APA or 7-ADCA, [illegible] steps is thus avoided, and investments in purification equipment for, for example, Ampicillin, Amoxicillin and Cephalexin are thus minimized as purification equipment former used for isolation of 6-APA [illegible] be used for isolation of Ampicillin, Amoxicillin and Cephalexin.

BSPR:
Advantageously, [illegible] high yield [illegible] this invention can be obtained [illegible]. Thus, [illegible] yield of Ampicillin was obtained [illegible].

BSPR:

A[illegible], [illegible] amino .beta.-lactam, for example, [illegible], 7-amino[illegible] acid, 7-amino[illegible] acid or 7-amino-3-[illegible]-3-cephem-4-carboxylic acid, with an acylating agent, for example, a derivative of D-phenylglycine or D-p-hydroxyphenylglycine.

BSPR:
In one aspect, this process is characterized in that the concentration of the starting amino .beta.-lactam in the reaction mixture is in the range from [illegible] mM, preferably above about [illegible] mM, more preferred above [illegible] mM, most preferred above about [illegible] mM. In another aspect, this process is characterized in that the initial concentration of the starting amino .beta.-lactam in the reaction mixture is in the range from about [illegible] about [illegible] mM, preferably above about [illegible] mM, more preferred above about [illegible] mM, most preferred above about 2[illegible] mM. In a still further aspect, this process is characterized in that the initial amount of the acylating agent, for example, the D-phenylglycine or D-p-hydroxyphenylglycine derivative, in the reaction mixture is above the solubility of said agent in the reaction mixture (preferably at least [illegible] above the solubility); or the initial amount of the acylating agent in the reaction mixture is above half of the solubility of said acylating agent plus the initial amount of the amino .beta.-lactam in the reaction mixture (preferably above the solubility of the acylating agent in the reaction mixture the initial amount of the amino .beta.-lactam). In a still further aspect, this invention relates to a process whereby the concentration of acylating agent plus the concentration of .beta.-lactam derivative in the reaction mixture is above about 45[illegible] mM, preferably above about 5[illegible] mM, more preferred above about 650 mM, even more preferred above about [illegible] mM). In an additional aspect, this invention relates to a process whereby the initial concentration of acylating agent in the reaction mixture is above about [illegible] mM, preferably above about [illegible] mM, more preferred above about [illegible] mM, even more preferred above about [illegible] mM.

DEPR:
Examples of .beta.-lactam derivatives that may be produced by the process of this invention are Ampicillin, Amoxicillin, Cefaclor, Cephalexin, Cephadroxil.

DEPR:
The acylating agent may be a derivative of D-phenylglycine or D-p-hydroxyphenylglycine such as a lower alkyl (methyl, ethyl, n-propyl or isopropyl) ester or an amide which is unsubstituted in the --CONH.sub.2 group. [illegible] The derivative may be used in the form of a salt, for example, the HCl salt or the H.sub.2 S.sub.4 salt. The acylating agent may be added in an active form or the active form may be formed in situ.

DEPR:
The enzyme to be used in the process of this invention may be any enzyme catalyzing the reaction in question. Such enzymes have been known [illegible]. Enzymes to be used are, for example, termed penicillin amidase or penicillin acylase and classified as E.C. 3.5.1.11. A number of microbial enzymes are known to have this activity, derived from for example Acetobacter, [illegible], [illegible], [illegible], Aeromonas (West German patent application having publication No. [illegible]), Pseudomonas (Austrian Patent No. [illegible]), Flavobacterium (Dutch patent application No. [illegible]), Aphanocladium, Cephalosporium (West German patent application having publication No. [illegible]), Acetobacter pasteurianum, Bacillus megaterium, Xanthomonas citrii (European patent application having publication No. [illegible]), Kluyvera citrophila (Agr. Biol. Chem. 37 (1973), [illegible]) and Escherichia coli (West German patent application having publication No. [illegible]). The Escherichia coli enzyme is commercially available. The enzyme also may be a so-called ampicillin hydrolase, acylase or amidase. In this [illegible]

DEPR:

[illegible] enzyme [illegible], for example, [illegible]. Immobilization may be [illegible]. Immobilized [illegible] enzyme is commercially available from Boehringer Mannheim GmbH, Germany, under the trade name Enzygel.

DEPR:
The solubility of the acylating agent such as the D-phenylglycine or D-p-hydroxyphenylglycine derivative will vary with the [illegible] of the [illegible] solubility of the [illegible] D-phenylglycine [illegible] typically approximately 4 [illegible]. However, the solubility is very dependent on the salt [illegible] in the solution, as well as on the pH value and the temperature of the solution. In some embodiments of the process of this invention, the initial reaction mixture is a slurry containing undissolved acylating agent and/or .beta.-lactam, which will dissolve partly or fully during the course of the reaction. The .beta.-lactam formed may precipitate during the reaction and, also, the hydrolysis products of the acylating agent such as D-phenylglycine and D-p-hydroxyphenylglycine, may precipitate. Hence, in many cases the reaction mixture will be a slurry throughout the reaction.

DEPR:
The amino .beta.-lactam, for example 6-APA or 7-ADCA, used in the process of this invention may be obtained by enzymatic hydrolysis of the fermented penicillins or cephalosporins, (for example penicillin V, penicillin G or cephalosporin C) or their ring enlarged analogues (for example V-DCA and G-DCA) or derivatives thereof followed by removal of the hydrolysis by-product, if desired (phenoxyacetic acid etc.). Advantageously, the crude solution can be used directly without further purification or dilution.

DEPR:
Generally, the reaction temperature of the process of this invention may vary between about [illegible].degree. C. and about 35.degree. C., is especially between about [illegible].degree. C. and about 30.degree. C. Temperatures in the range about [illegible].degree.-30.degree. C. may be preferred for convenient operation. The suitable pH value depends on the type and purity of enzyme. Using Escherichia coli enzyme, the pH value is typically in the range from about 5.5 through about 7.[illegible], preferably in the range from about [illegible] through about 7. For the preparation of Amoxicillin, a pH value in the range from about 5.5 through about [illegible] is preferred. [illegible] of the pH value may be used. Suitable reaction times are from several minutes to several hours, in particular from about [illegible] to about [illegible] hours. Suitable enzyme concentrations may be from about [illegible] ml to about [illegible] ml [illegible] unit of enzyme activity, see below).

DEPR:
Using the process according to this invention, extraordinary high yields can be obtained. The high yields are obtained using the teachings of this invention and properly selecting the concentration of the acylating agent, the ratio between the concentration of acylating agent and the starting amino .beta.-lactam, the pH value and the enzyme.

DEPR:
[illegible] enzyme that hydrolyses per minute [illegible] penicillin [illegible] penicillin [illegible] sodium phosphate buffer, pH value [illegible], [illegible].degree. C.

DEPR:
[illegible] mM 6-APA and D-[illegible] in a concentration as indicated in Table [illegible] adjusted to pH value [illegible] and equilibrated at [illegible].degree. C. and [illegible]

DEPR:

[illegible] in Example [illegible], only [illegible] mM [illegible] is used instead of 6-APA. [illegible] and the max. yields [illegible] shown in Table [illegible].

[illegible]:
[illegible] mM 6-APA and [illegible] mM D-PGM sulphate salt are adjusted to a pH value as indicated in Table [illegible], and the enzymatic synthesis is carried out at [illegible].degree. C. and pH [illegible], total volume [illegible] ml and [illegible] U soluble enzyme from [illegible].

[illegible]:
Starting with [illegible] mM 6-APA and [illegible] mM D-PGA at pH value [illegible] and [illegible] soluble enzyme from Escherichia coli (total volume: [illegible] ml) and running the synthesis at temperatures as indicated in Table 4, the maximal yields of Ampicillin obtained are shown in Table 4.

[illegible]:
This example was performed analagously with Example [illegible] using D-PGM instead of D-PGA. The maximum yields of Ampicillin obtained are as stated in Table [illegible].

[illegible]:
Pen V [illegible] filtration, extraction [illegible] acetate and [illegible] aqueous phase resulting in a solution [illegible] weight/volume) pen V is hydrolysed by Semacylase.TM. (Immobilized pen V acylase from Novo Nordisk A/S) at a pH value of 7.0. The phenoxyacetic acid is removed by extraction and to the resulting 6-APA (150 mM) solution, containing minor amounts of biproducts from degraded pen V and 6-APA, is added 45 U/ml soluble enzyme from Escherichia coli and D-PGA (to a final Concentration of [illegible] mM). The pH value is adjusted to 6.4 and the reaction is allowed to proceed at [illegible].degree. C. keeping the pH value constant.

[illegible]:
Under these conditions a total of 1[illegible] mmole of Ampicillin ([illegible]) is formed per liter of reaction volume.

[illegible]:
[illegible] mg of immobilized enzyme is suspended ad 10 ml with water. The enzyme solution was mixed with a solution of 6-APA and D-PGA to a total volume of 25 ml the resulting mixture containing [illegible] mM 6-APA and [illegible] mM D-PGA, having pH value [illegible] at room temperature. The synthesis reaction was allowed [illegible] hours after which [illegible] of the 6-APA was converted to Ampicillin.

[illegible]:
A mixture of 968 mg 6-APA and 3718 mg HPGA in water is adjusted to pH 6.2 at [illegible].degree. C. and [illegible] U soluble penicillin G acylase from E. coli is added to a final volume of [illegible] ml. The synthesis is allowed to proceed at constant temperature, using [illegible] sulphuric acid to keep the pH at [illegible]. After [illegible] hours the reaction mixture contained [illegible] mM Amoxicillin, corresponding to a yield of [illegible] based on the 6-APA consumption.

[illegible]:
[illegible] U soluble penicillin G acylase from E. coli is added to a mixture of 6-APA and HPGA ([illegible] mM and [illegible] mM final concentration, respectively) in water at pH [illegible] and [illegible].degree. C. After reacting for [illegible] hours keeping the temperature and pH constant using [illegible] sulphuric acid for the titration, 1[illegible] mM Amoxicillin was produced ([illegible] yield) based on HPLC-analysis.

[illegible]:
Starting with [illegible] mM 6-APA, [illegible] mM HPGA, [illegible] U soluble penicillin G acylase from E. coli, 14[illegible] mM Amoxicillin ([illegible]) was produced after 4 hours, when the reaction was carried out at pH [illegible] and [illegible].degree. C.

[illegible]:
Same conditions as described in example [illegible], using [illegible] mM 6-APA and 4[illegible] mM HPGA

[illegible]

[illegible]:
6-APA is 6-aminopenicillanic acid, 7-ADCA is 7-aminodesacetoxycephalosporanic acid, D-PGA is D-phenylglycinamide, DPGM is D-phenylglycin methyl ester, [illegible] is 7-phenoxyacetamidodesacetoxycephalosporanic acid, [illegible] is 7-phenylacetamidodesacetoxycephalosporanic acid and HPGA is [illegible].

[illegible]:
[illegible]

[illegible]:
Enzymatic synthesis of Ampicillin.

[illegible]:
Enzymatic synthesis of Cephalexin.

[illegible]:
Enzymatic synthesis of Amoxicillin.

DEPU:
Enzymatic synthesis of Amoxicillin.

DEPU:
Enzymatic synthesis of Amoxicillin.

DEPU:
Enzymatic synthesis of Amoxicillin.

[illegible]:
Retention times in minutes: 4.1 (D-PG); 6.3 (7-ADCA); 8.1 (6-APA); 9.1 (D-PGA); 1[illegible].4 (Cephalexin); 13.9 (Ampicillin); 18 (D-PGM).

DEPU:
Retention times in minutes: 2.5 (D-p-hydroxyphenylglycine); 3.3 (HPGA); 5.4 (6-APA); 13.2 (Amoxicillin).

[illegible]:
TABLE [illegible] maximum yield reaction time, mM
[illegible]

DETL:
TABLE 5 D-PGM, Ampicillin formed,
reaction time, mM hours 270 74 12 [illegible]
[illegible]

[illegible]:
1. In the process for the preparation of a .beta.-lactam amide comprising [illegible] selected from the group consisting of a penicillin, [illegible]desacetoxycephalosporanic acid, 7-aminocephalosporanic acid and [illegible] to an enzymatic reaction with an acylating agent in a reaction mixture containing less than [illegible] volume/volume [illegible], [illegible] the .beta.-lactam amide is amoxicillin and the acylating agent is D-.alpha.-(p-hydroxy-phenyl) glycine methyl ester, the improvement comprising using a concentration of the acylating agent and the .beta.-lactam amide in the reaction mixture greater than 4[illegible] mM and the enzyme is derived from a microorganism selected from the group consisting of Escherichia coli, [illegible], [illegible], Kluyvera citrophila, or Bacillus [illegible].

[illegible]:
[illegible]. A process according to claim [illegible], wherein the amino .beta.-lactam is 6-aminopenicillanic acid, 7-aminodesacetoxycephalosporanic acid,

[illegible]

CLPR:
[illegible]. A process according to claim 1 or 2, wherein the acylating agent is [illegible] or D-p-hydroxyphenylglycine or derivatives thereof.

CLPR:
[illegible]

CLPR:
1[illegible]. A process according to claim 1 or 2, wherein the concentration of the amino .beta.-lactam in the reaction mixture when the enzymatic reaction starts is in the range from about [illegible] to about [illegible] mM.

CLPR:
1[illegible]. A process according to claim 1 or 2, wherein the concentration of acylating agent in the reaction mixture when the enzymatic reaction starts is greater than [illegible] mM.

CLPR:
[illegible]. A process according to claim 1[illegible], wherein the concentration of the acylating agent in the reaction mixture when the enzymatic reaction starts is greater than [illegible] mM.

CLPR:
[illegible]. A process according to claim 1 or 2, wherein the amount of the acylating agent in the reaction mixture when the enzymatic reaction starts is above the solubility of the agent in the reaction mixture.

CLPR:
[illegible]. A process according to claim 1 or 2, wherein the amount of the acylating agent in the starting reaction mixture is greater than half the amount of said agent which is soluble in the reaction mixture plus the amount of the amino .beta.-lactam in the reaction mixture when the enzymatic reaction starts.

CLPR:
[illegible]. A process according to claim 1 or 2, wherein the enzyme used is classified as EC [illegible].

CLPR:
[illegible]

CLPR:
[illegible]. A process according to claim 1 or 2, wherein an enzyme in reusable form is used.

CLPR:
[illegible]